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The Effect of Nitrogen Mustards on the Viscosity of Thymonucleate*

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The nitrogen mustards are compounds which have definite cytotoxic effects. The exact mechanism for this action is not known but some investigators have assumed that nuclear function is inhibited or destroyed. The present investigation is concerned with the effect of nitrogen mustards on the viscosity of thymus nucleate.

METHODS

The nitrogen mustards used in these experiments were the hydrochlorides of ethyl bis (β chloroethyl) amine, methyl bis (β chloroethyl) amine, and tris (β chloroethyl) amine.

Sodium nucleate was prepared from calf thymus by a modification of Hammarsten's procedure (5). After the preliminary extraction, filtration, and addition of $CaCl_2$, a precipitate was obtained by bringing the final ethanol concentration to 15 per cent. The precipitate was kept in the refrigerator overnight, and separated by centrifuging and washed with 15 per cent ethanol. The washed precipitate was dissolved in a saturated NaCl solution and allowed to stand in the refrigerator for 3 days. The solution was filtered through "Hyflo Super-Cel" under vacuum in a Buchner funnel. The sodium thymonucleate was precipitated by adding 95 per cent ethanol and was washed and redissolved in H_2O. It was reprecipitated and washed with ethanol and ether and dried in vacuo over P_2O_5. Despite the uniformity in procedure, the viscosity of the various batches of thymonucleate varied considerably.

The viscosity measurements were made according to the method described by Bingham and Jackson (1). A pressure of 20 cm. H_2O was used and the viscosimeter was kept in a constant temperature bath at 30° C. No attempt was made to express the values in terms of absolute viscosity.

Most of the experiments were performed by diluting 10 ml. of a 1 per cent thymonucleate solution with an equal quantity of buffer solution; the agent was either added to this mixture in solid form or in solution.

TABLE I: EFFECT OF VARIOUS CONCENTRATIONS OF NITROGEN MUSTARDS ON THE VISCOSITY OF A THYMONUCLEATE SOLUTION

	Minutes after mixing				
	10	30	60	90	
Agent *	Seconds				pH †
Control	367	375	383	390	6.0
0.002 M					
1	369	366	362	355	6.0
2	368	366	366	360	6.0
3	340	316	307	302	5.9
0.004 M					
1	369	364	355	340	5.9
2	369	366	360	350	5.9
3	310	251	233	224	5.7
0.01 M					
1	369	362	331	284	5.7
2	369	364	340	312	5.7
3	227	165	146	132	5.6

* The agents designated as follows:
(1) Ethyl bis (β chloroethyl) amine,
(2) Methyl bis (β chloroethyl) amine, and
(3) Tris (β chloroethyl) amine.
† Values for pH obtained after final viscosity reading.

RESULTS

Effect of concentration of nitrogen mustards.—To equal volumes (10 ml.) of a 1 per cent thymonucleate solution and an acetate buffer of pH 6.0 and ionic strength of 0.3, 1 ml. of an aqueous solution of the nitrogen mustards was added. The nitrogen mustards added were present so that the final molar concentrations were 0.01, 0.004, and 0.002.

The results are shown in Table I. At all concentrations the tris (β chloroethyl) amine causes appreciable

* This work was done under contract with the Medical Division of the Chemical Warfare Service.

decreases in viscosity of the nucleate solution. The effect of the remaining two agents is comparatively small.

Slight changes are seen in the hydrogen ion concentration after addition of the nitrogen mustard in these and in the following experiments. According to Vilbrandt and Tennent (7) small changes in pH below 5.0 and above 9.0 may influence the viscosity of nucleic acid solutions considerably. These findings were confirmed in this laboratory for the ionic strength (1 per cent NaCl) used by the investigators. It should be stated that increasing the ionic strength is responsible for more noticeable changes in viscosity within given ranges in pH. The differences in pH noted in Tables I to III are not sufficiently great to produce the changes noted after addition of nitrogen mustards.

TABLE II: EFFECT OF IONIC STRENGTH ON THE DEPOLYMERIZING ACTION OF TRIS (β CHLOROETHYL) AMINE ON THYMONUCLEATE

	Ionic strength	Minutes after mixing: 15 Seconds	30 Seconds	60 Seconds	pH †
Control	1.50	335	348	368	6.0
Agent		355	366	380	5.9
Control	1.00	310	322	330	6.0
Agent		294	297	305	5.9
Control	0.50	322	328	338	6.0
Agent		288	286	286	5.9
Control	0.30	306	308	314	6.0
Agent		267	258	250	5.9
Control	0.15	292	292	290	6.0
Agent		230	210	197	5.8
Control	0.06	300	298	295	6.0
Agent		208	183	164	5.8

† See this footnote on Table I.

Effect of ionic strength.—Since tris (β chloroethyl) amine caused an immediate decrease in the viscosity of thymonucleate, this agent was used to determine the effect of salt concentration.

Acetate buffers with an initial pH of 6.0 were prepared with ionic strengths of 1.5, 1.0, 0.5, 0.3, 0.15, and 0.06. The control was prepared by mixing 10 ml. of nucleate solution and 10 ml. of the respective buffers and 1 ml. of H_2O. Tris (β chloroethyl) amine hydrochloride (10 mgm.) was dissolved in 1 ml. of H_2O and added to the nucleic acid mixture immediately. The results are shown in Table II.

A lowering of the ionic strength results in a decreased viscosity in the control groups. The viscosity becomes progressively less for a given amount of agent as the ionic strength decreases.

Effects of compounds structurally related to the sulfur and nitrogen mustards.—To a mixture of 10 ml. thymonucleate (1 per cent) and 10 cc. acetate buffer having a final pH 6.0 and ionic strength of 0.15, the following compounds dissolved in 1 ml. H_2O were added to yield equimolar concentrations (1×10^{-4}).

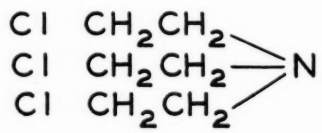
a. choline chloride	$(HOCH_2CH_2N(CH_3)_3)Cl$
b. guanidine hydrochloride	$NH = C(NH_2)_2HCl$
c. triethanol amine	$N(CH_2CH_2OH)_3$
d. thiodiglycol	$S(CH_2CH_2OH)_2$
e. dichloroethyl sulfide	$S(CH_2CH_2Cl)_2$
f. tris (β chloroethyl) amine	$N(CH_2CH_2Cl)_3$

The control solution was similarly prepared, but no agent was added.

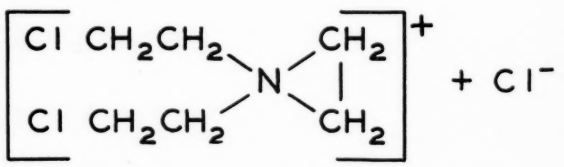
In Fig. 1, it is seen that the compounds a, b, c, and d increased viscosity above that of the control. Sulfur mustard e causes an appreciable initial decrease in viscosity which remains constant on standing. The nitrogen mustard f decreases the viscosity as previously shown. The importance of the chloroethyl group is apparent in these experiments.

Effect of transformation products.—Nitrogen mustards in aqueous solutions undergo characteristic intramolecular rearrangements to form a number of compounds. The rate of formation of these transformation products differs for each compound and is dependent on the hydrogen ion concentration and the inorganic and organic substances present in the solution. According to Gilman, Goodman, and Phillips (2), the various transformation products of a given nitrogen mustard differ in their pharmacodynamic action and toxicity.

The active physiological product formed appears to be an ethylene-imine ring derivative. For example, the compound tris (β chloroethyl) amine



undergoes a change to form the first imine ring.



Each chloroethyl radical is capable of forming an imine ring. The rate of formation of these rings can be slowed by using a $NaHCO_3$:NaOH solution of about pH 9.0 so that mixtures can be obtained in which a given ring is predominant (7).

The 3 nitrogen mustards were dissolved in the alkaline buffer and were allowed to stand at room temperature for varying lengths of time until the desired ring was present. A 1 per cent nucleate solution was mixed with an equal amount (10 ml.) of the alkaline solution of nitrogen mustard which contained 20 mgm. of the

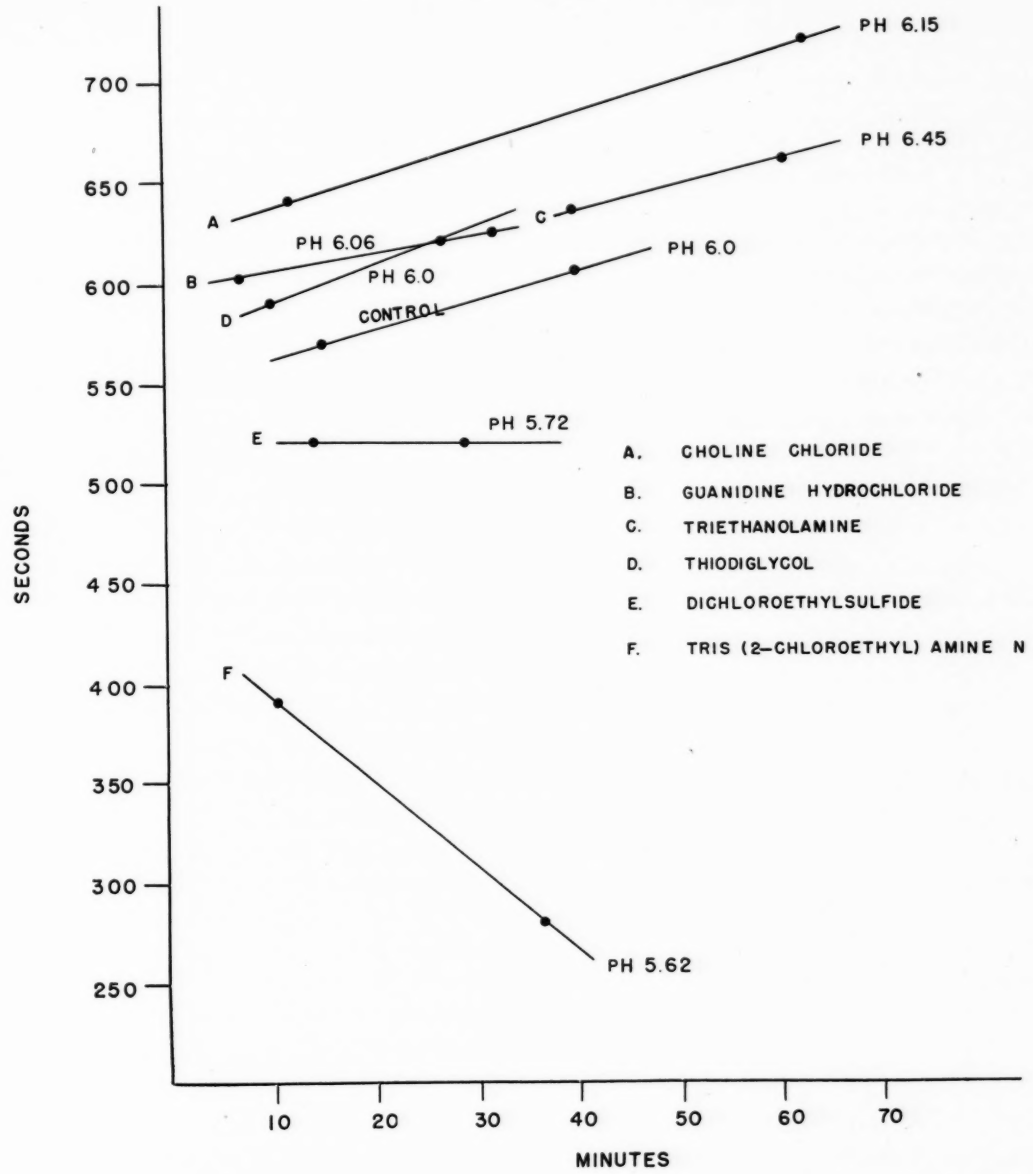


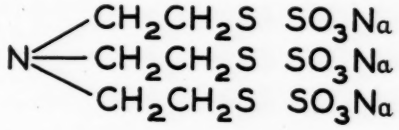
FIG. 1.—Effect of sulfur mustard and a nitrogen mustard and related compounds on the viscosity of a nucleic acid solution.

respective agents. After mixing, the pH of the solution decreased to about 8.0.

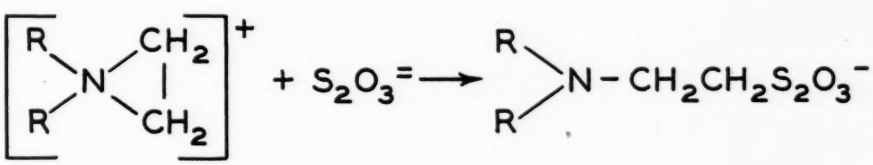
The results for a typical set of experiments are shown in Fig. 2. In each case the solution containing a preponderance of the 1st imine ring has the greatest effect in reducing the viscosity of the nucleic acid. In the case of the tris (β chloroethyl) amine, the 2nd imine ring is more effective than the 3rd imine ring.

In the presence of thiosulfate a relatively stable end product is formed.

A molecule of thiosulfate is required for each imine ring formed. Thus, in the case of the tris (β chloroethyl) amine the final product is



which is a stable, non-toxic compound.



Equal volumes (10 ml.) of 1 per cent nucleic acid and phosphate buffer (pH 7.5, ionic strength 0.3) solutions which contained various amounts of sodium thiosulfate were mixed. Tris (β chloroethyl) amine hydrochloride (50 mgm.) was dissolved in 1 ml. H_2O and added. In a preliminary experiment in which 3 moles of thiosulfate were added to react with the 3 imine rings of the agent, the usual decrease in viscosity was completely inhibited.

In Fig. 3, the effect of varying the amounts of thiosulfate on viscosity is shown. The reduction in available thiosulfate is accompanied by a decreased viscosity of thymonucleate. This appears to be proof that the imine ring is directly concerned with depolymerization of nucleic acid.

Table III: The Effect of Thiocyanate on the Depolymerizing Effect of Tris (β chloroethyl) Amine on Thymonucleate

NaSCN mgm.	Agent mgm.	Minutes after mixing: 10 Seconds	30 Seconds	60 Seconds	pH †
50.3	0	271	271	271	7.1
75.0	50	233	205	190	6.7
50.3	50	232	204	180	6.7
30.0	50	162	135	110	6.7
20.0	50	159	128	120	6.7
0.0	50	139	119	105	6.7

† See this footnote on Table I.

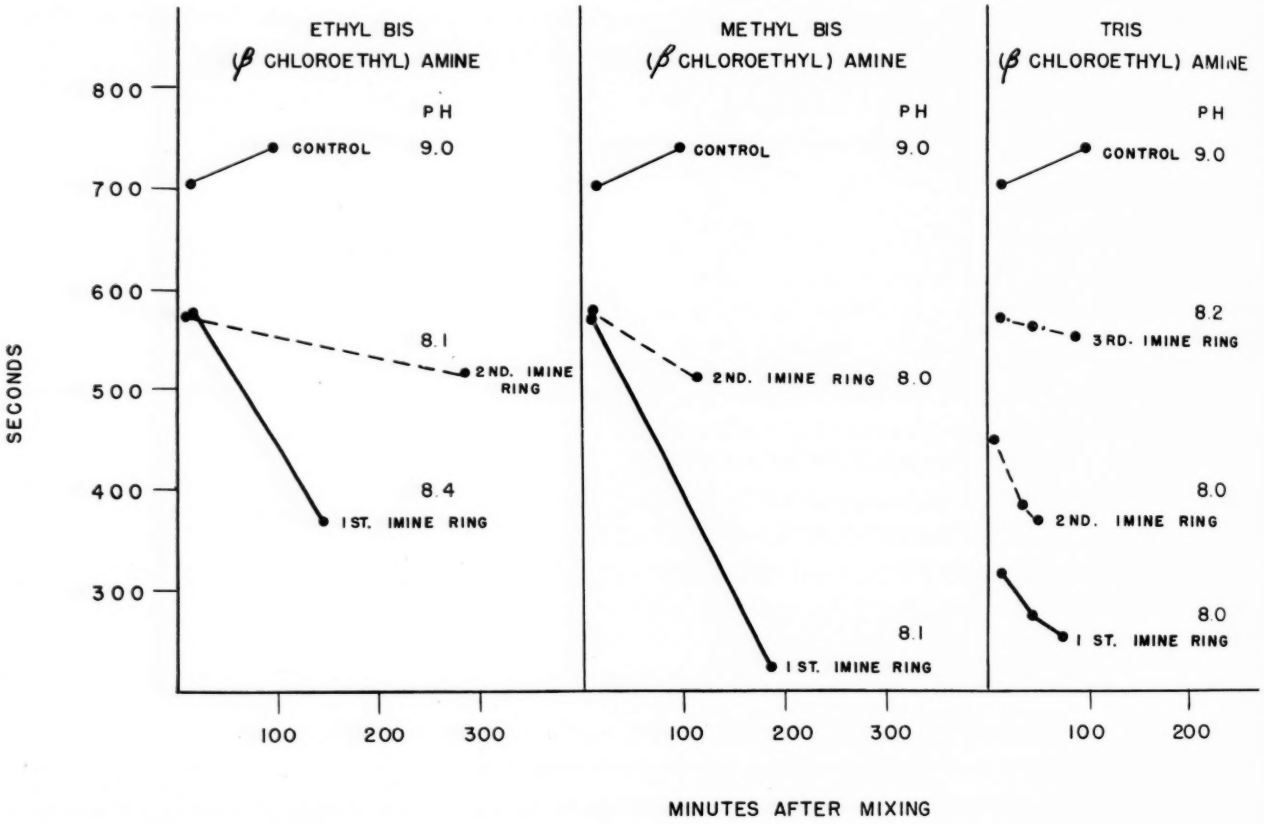


Fig. 2.—Effect of transformation products of three nitrogen mustards on the viscosity of a nucleic acid solution.

Sodium thiocyanate is known to react with the imine ring. However, the competition of thiocyanate for the imine ring is not as great as that of thiosulfate. According to theory, 3 moles of NaSCN should react with and form stable salts with the 3 potential imine rings of tris (β chloroethyl) amine. It should require 50.3 mgm. NaSCN to neutralize 50 mgm. of the hydrochloride. The results of adding varying amounts of SCN to a thymonucleate mixture similar to that described for thiosulfate are shown in Table III. This ion is partially effective in reducing the depolymerizing effect of the nitrogen mustards. It will be noted that excessive amounts of thiocyanate (75 mgm.) do not prevent the decrease in the viscosity of thymonucleate solutions.

DISCUSSION

There is no doubt that the sodium thymonucleate is a highly polymerized structure made up of many molecules of tetranucleotides (4). An enzyme such as desoxyribonuclease (6) causes a rapid depolymerization with an accompanying decrease in viscosity. No compound is as effective as this enzyme. However, as

far as can be ascertained, transformation products of the nitrogen mustards are more effective than any other chemical compound in depolymerizing nucleic acid.

dilution of small amounts of the injected agent in the blood stream. Gilman and Philips (3), in a recent review, stressed the high degree of specificity of these agents on lymphoid tissue, bone marrow, and intestinal

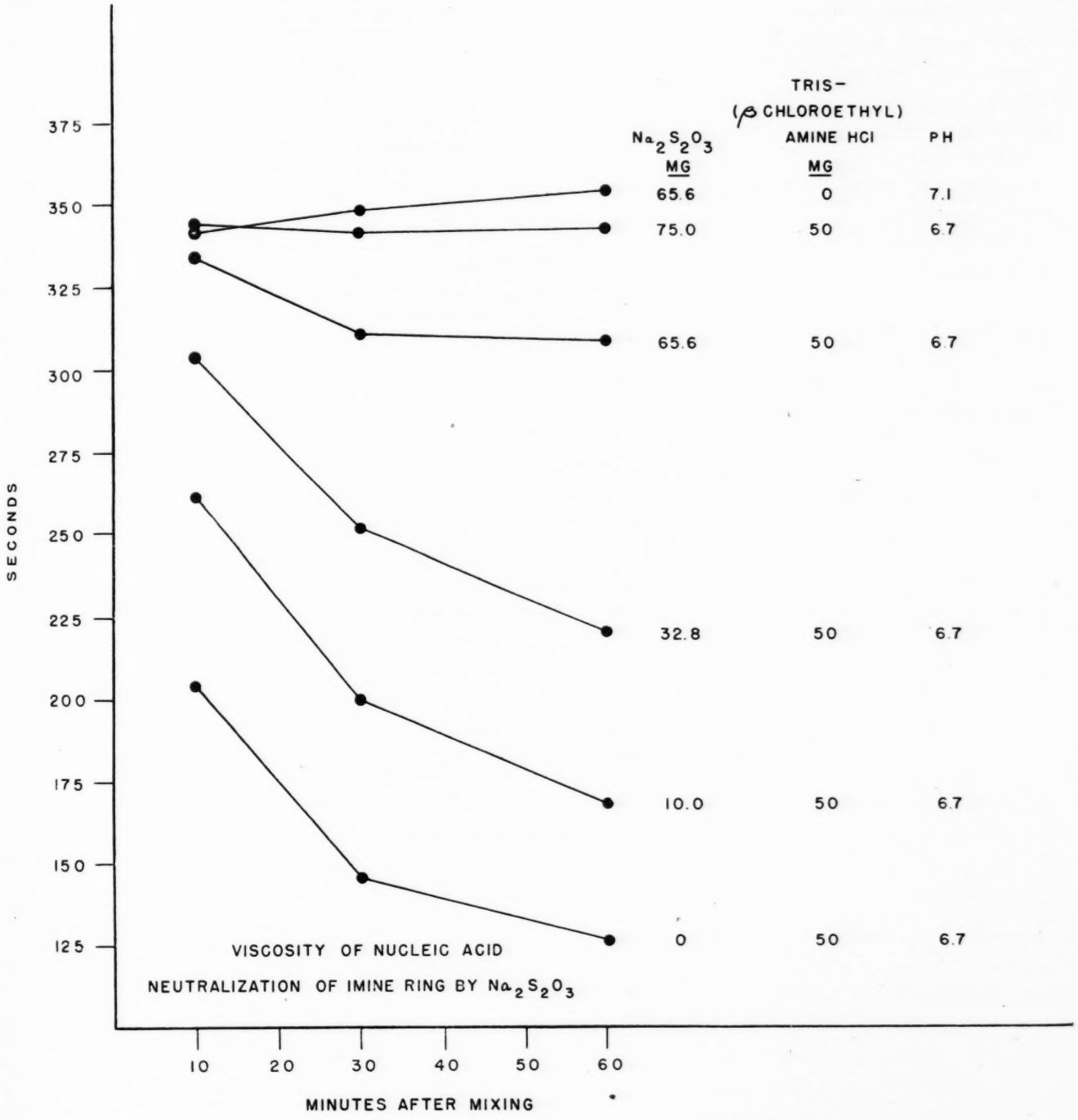


FIG. 3.—Neutralization of transformation products of a nitrogen mustard by sodium thiosulfate.

The toxicity of the nitrogen mustards may be explained in part by the action of the transformation products on the nucleoproteins of the cells. The objection to this hypothesis could be based on the high

mucosa. They also pointed out that the mustards can exhibit a primary nucleotoxic action. These agents have been studied for their therapeutic effects in the treatment of neoplasms of lymphoid tissue.

SUMMARY

1. The influence of 3 nitrogen mustards, ethyl bis (β chloroethyl) amine, methyl bis (β chloroethyl) amine, tris (β chloroethyl) amine, on the viscosity of thymus nucleate has been investigated.

2. The viscosity of thymus nucleate solutions is decreased by these mustards. The greatest decreases in viscosity are noted in solutions of low ionic strength. The degree and rate of change in viscosity depends on the formation of active transformation products.

3. Evidence is presented to show the importance of the chloroethyl radical in the depolymerizing action of the mustards.

4. The ethylene-imine ring transformation product of the nitrogen mustards appears to be responsible for the depolymerizing effect. This effect is most pronounced when the first imine ring predominates.

5. In the presence of thiosulfate, the effect of the ethylene-imine rings is completely inhibited. Thiocyanate partially inhibits the action of these rings.

ACKNOWLEDGMENT

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The Effect of Nitrogen Mustards upon the Ultraviolet Absorption Spectrum of Thymonucleate, Uracil and Purines*

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(Received for publication May 8, 1946)

In a previous paper (2) it was demonstrated that nitrogen mustards caused a considerable decrease in the viscosity of sodium thymonucleate solutions. The present work was undertaken to determine whether this effect was due to an interaction of the nitrogen mustards with thymonucleate or some of its constituents.

An interaction between nitrogen mustards and thymonucleate was demonstrated but could not be correlated with the effect on viscosity.

EXPERIMENTAL

Sodium thymonucleate.—A 2 per cent solution of sodium thymonucleate (2) was diluted with an equal volume of an acetate buffer (pH 6.0; ionic strength 0.2). Nitrogen mustards were added to 50 ml. of this nucleic acid-acetate mixture and stirred for an hour at room temperature. A 2 ml. aliquot was diluted to 200 ml. with water and the ultraviolet absorption spectrum was determined in a Beckman spectrophotometer. The control solution was treated similarly but no agent was added.

In preliminary experiments it was found that tris (β chloroethyl) amine caused a shift in the absorption spectrum. Equimolar concentrations of ethyl bis (β chloroethyl) amine and methyl bis (β chloroethyl) amine did not cause a similar shift. It is assumed that the negative effect of the 2 latter compounds was due to the slow formation of active transformation products. Consequently, tris (β chloroethyl) amine was used for all experiments.

To 50 ml. of the nucleate-acetate buffer mixture were added varying amounts of tris (β chloroethyl) amine hydrochloride and the procedure described above was followed. The absorption spectra are shown in Fig. 1. The maximum absorptions for the control and the 15, 30, 63, and 125 mgm. groups are 259, 259, 260, 262, and 263 mμ, respectively. The shift in the spectrum towards the longer wave lengths becomes more pronounced with increased concentrations of the agent. No change is observed after the addition of 15 mgm. of the nitrogen mustard. Pronounced changes in viscosity of nucleic acid are seen at much lower

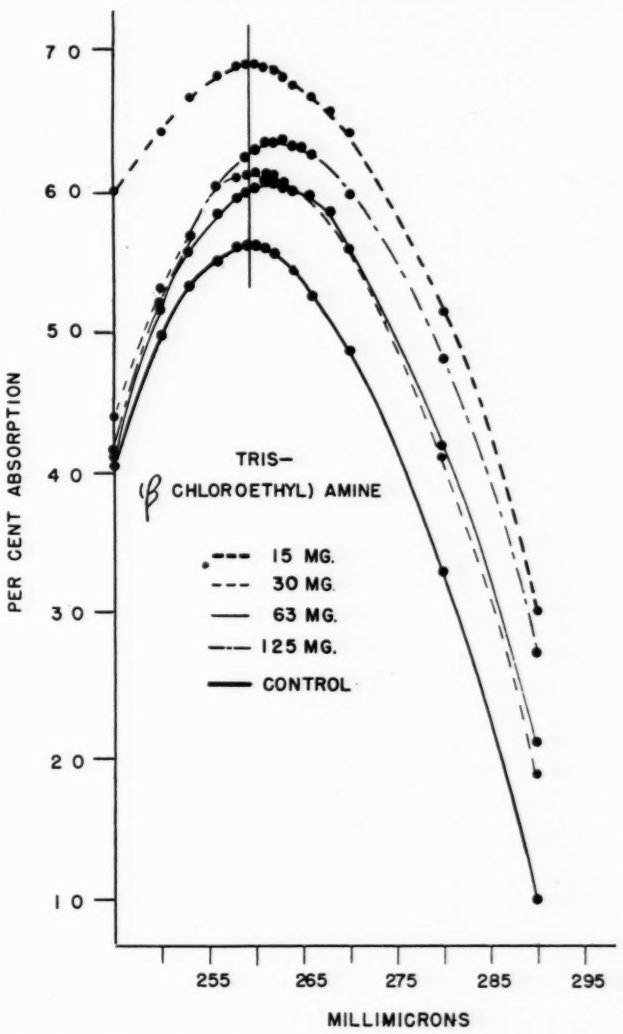

FIG. 1.—Effect of varying amounts of a nitrogen mustard on absorption spectrum of thymonucleate.

concentrations (2). Since large amounts of the agent are necessary to produce a shift in the spectrum, it is assumed that this phenomenon is not associated with the depolymerizing effect of nitrogen mustards. It is

* This work was done under contract with the Medical Division of the Chemical Warfare Service.

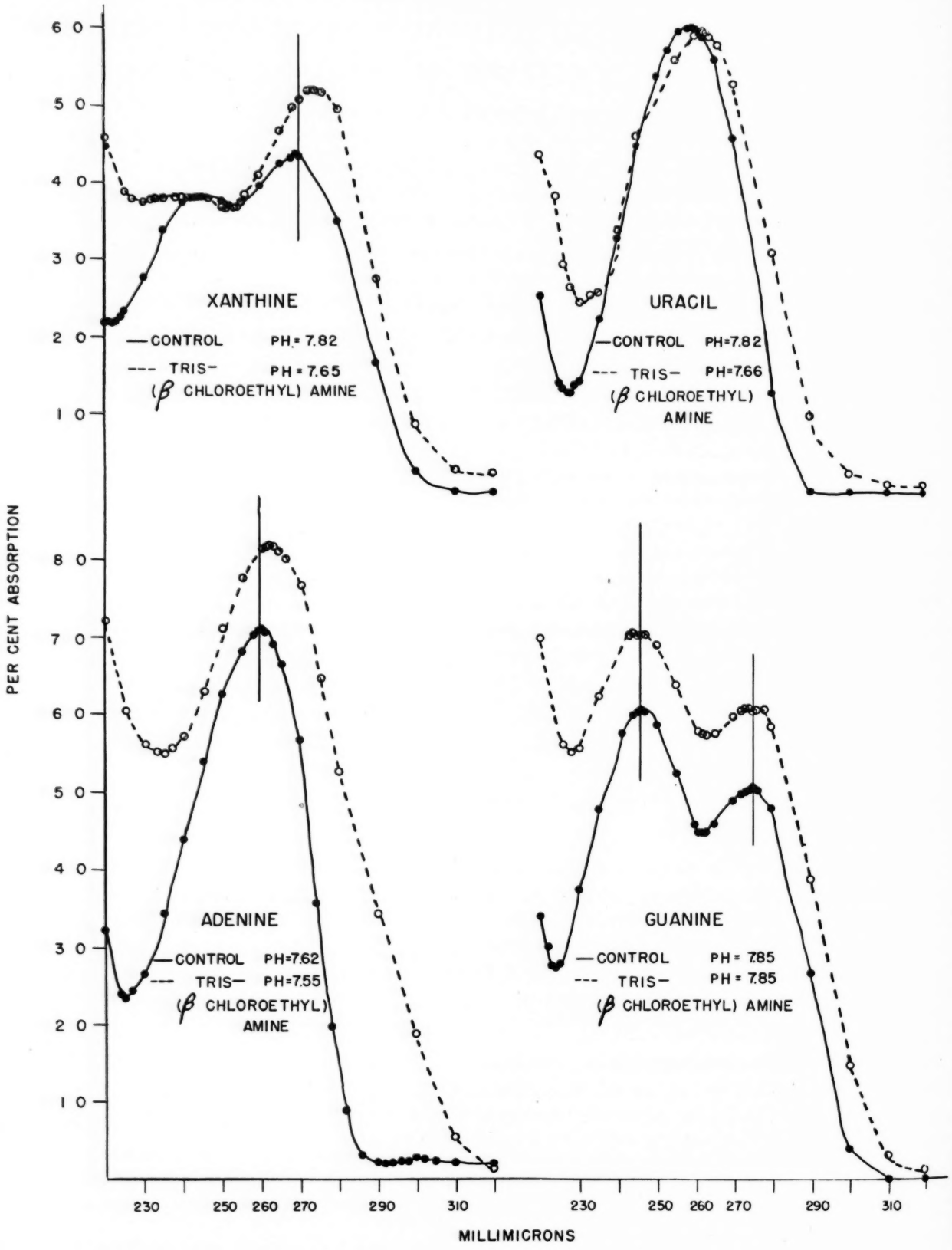

FIG. 2.—Effect of a nitrogen mustard on absorption spectrum.

well known that weighting a molecule with an aliphatic hydrocarbon chain causes a similar shift in absorption.

Purines and pyrimidine.—Solutions of adenine sulfate, $2H_2O$ (0.1 per cent); guanine hydrochloride, $2H_2O$ (0.1 per cent); xanthine (0.06 per cent); and uracil (0.06 per cent) were prepared in a borate buffer of pH 9.2. To 50 ml. of each of the above solutions were added 50 mgm. of tris (β chloroethyl) amine hydrochloride and the mixture was stirred for 1½ hours. During the stirring, the pH of the solution was maintained between pH 8 and 10 by adding ammonium hydroxide. This alkaline reaction was necessary to keep the materials in solution. To a 4 ml. aliquot, 15 ml. of phosphate buffer (pH 7.4) were added and brought to 100 ml. with water. The absorption spectra of these dilute solutions were determined.

TABLE I: EFFECT OF TRIS (β CHLOROETHYL) AMINE ON THE ULTRAVIOLET ABSORPTION SPECTRUM OF 3 PURINES AND A PYRIMIDINE

	Control	Tris (β chloroethyl) amine
	Maximum absorption, mμ	Maximum absorption, mμ
Adenine	260	262
Guanine	246, 272.5	244, 246, 274, 278
Xanthine	245, 269	273
Uracil	259	262

The absorption spectra of the control and treated samples are shown in Fig. 2. The detailed data are given in Table I. It is clear that the guanine-nitrogen mustard mixture yields a new spectrum, characterized by the appearance of two new peaks. The changes occurring after treating xanthine are pronounced, since one peak disappears and the other is shifted. There is a definite shift of the spectrum to longer wave lengths in the case of adenine and uracil.

DISCUSSION

The nitrogen mustards are capable of reacting with amino acids, peptides, proteins, pyridine compounds, adenosine, thiamin and other compounds such as inorganic and organic phosphates (1). Evidence is presented in these experiments that purines and pyrimidines also react with these agents.

At present no procedure is available to determine the group or groups in nucleic acid which react with nitrogen mustard to cause changes in the ultraviolet absorption spectrum.

SUMMARY

Spectrophotometric evidence is presented to show that the nitrogen mustard, tris (β chloroethyl) amine, reacts with sodium thymonucleate, adenine, guanine, xanthine, and uracil.

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Induction of Neoplasia *in Vitro* with a Virus

Experiments with Rabbit Skin Grown in Tissue Culture and Treated with Shope Papilloma Virus*

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Since neoplasia has been induced in tissue culture by means of a chemical carcinogen (5), the question arises whether similar neoplastic transformation of cells *in vitro* can be brought about by other types of carcinogens, such as viruses.

This question was answered by successfully growing epidermis of the domestic rabbit in tissue culture and then inoculating it with the Shope papilloma virus. The experiments to be reported show that under these conditions, neoplastic transformation of epithelial cells occurs *in vitro*.

MATERIAL AND METHODS

The method of tissue culture consisted in embedding skin fragments in streaks of plasma on the side of test tubes. This method as previously described (3) was slightly modified because of difficulties inherent in cultivating skin epithelium.

The back of a domestic rabbit was shaved with a razor as closely as possible. The exposed skin was then scrubbed with warm water and soap daily for 4 days. on the fourth day the skin was washed with 95% alcohol, after which an elliptical piece about 3 cm. long was removed surgically and placed in physiological saline solution. No attempt was made to separate the epidermis from the underlying connective tissue. The skin was cut into fragments 2-3 mm. in diameter and these were embedded in a streak of chicken plasma in an ordinary test tube. The plasma was clotted with embryo extract, and a fluid medium consisting of human umbilical cord serum diluted with saline solution was added. In the saline solution was dissolved aspartic acid (0.3%), penicillin (1500 units per cc.), and sulfanilamide (2.0%). The final fluid medium, for each tube, consisted of 10 drops of serum, 10 drops of salt solution containing the aspartic acid and penicillin, and 10 drops of salt solution containing the sulfanilamide. The tubes were then placed in a rotator (4) housed in an incubator at 37° C.

To such cultures papilloma virus was added. Papillomas [1] from cotton-tail rabbits were ground in a mortar with sand to form a smooth paste. The paste was extracted with 10 volumes of physiological salt solution for 12 hrs. This suspension was then centrifuged and the supernatant fluid passed through a Berkefeld N filter. The resulting clear, sterile, cell-free filtrate was tested for potency in the skin of domestic rabbits before using in the tissue cultures. Usually 6 drops of active virus preparation were added to each culture tube. The fluid medium was changed every third or fourth day.

RESULTS

Cultures without papilloma virus, after 3 to 5 days, showed small clusters of squamous epithelial cells at the edges of the skin fragments. These clusters slowly enlarged to form small sheets of cells (Fig. 1). Growth was never rapid or luxuriant and nearly ceased after a week or 10 days, with slow deterioration of the cultures thereafter.

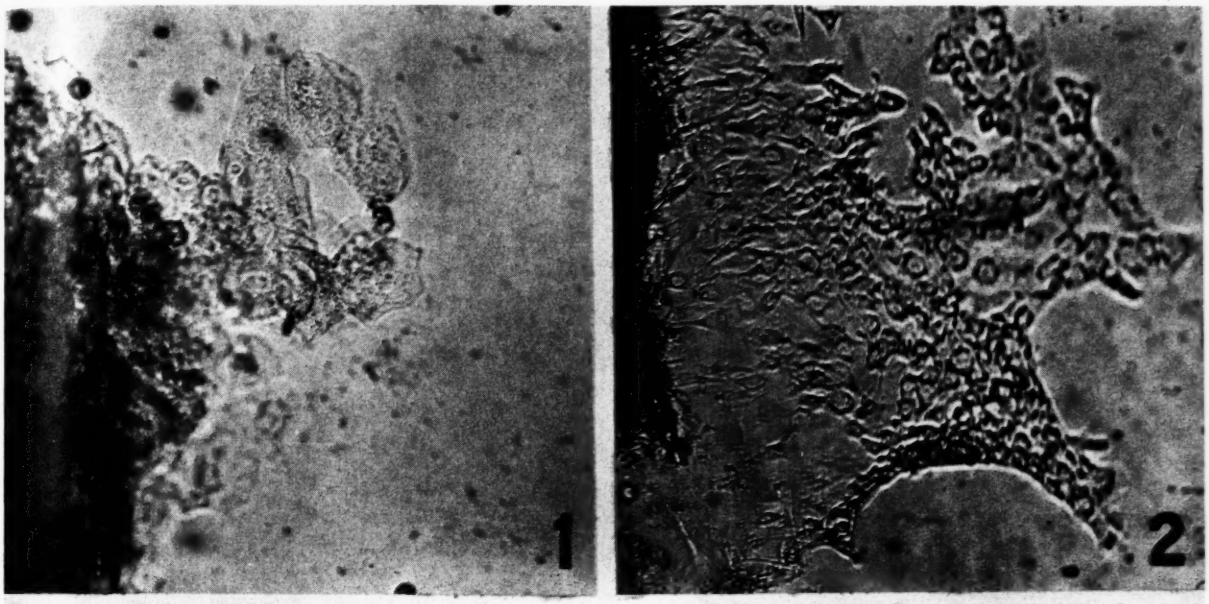
In contrast, when papilloma virus was added to such cultures a burst of epithelial proliferation followed within one or two days and vigorous growth persisted for 2 to 3 weeks (Fig. 2). Much larger sheets of epithelial cells were formed which eventually broke up into smaller clusters. Cytological studies upon untreated and upon virus-treated cultures were made in hanging drops to allow the use of higher power lenses. No significant changes were noted in the cells from virus-treated cultures.

The increased growth activity of the cultures following introduction of the virus suggested the probability that neoplasia was being induced *in vitro*. To test this probability further, explants were removed from the tubes, washed in saline and implanted in the livers of domestic rabbits as follows:

Virus-treated cultures of skin were implanted in the liver of the same domestic rabbit from which the skin

* This investigation was aided by a grant from the Cancer Research Division of The Donner Foundation.

[1] The papillomas used as a source of virus were obtained through the kindness of Drs. Richard Shope and Peyton Rous, of the Rockefeller Institute for Medical Research, and Dr. Joseph Beard, of Duke University.



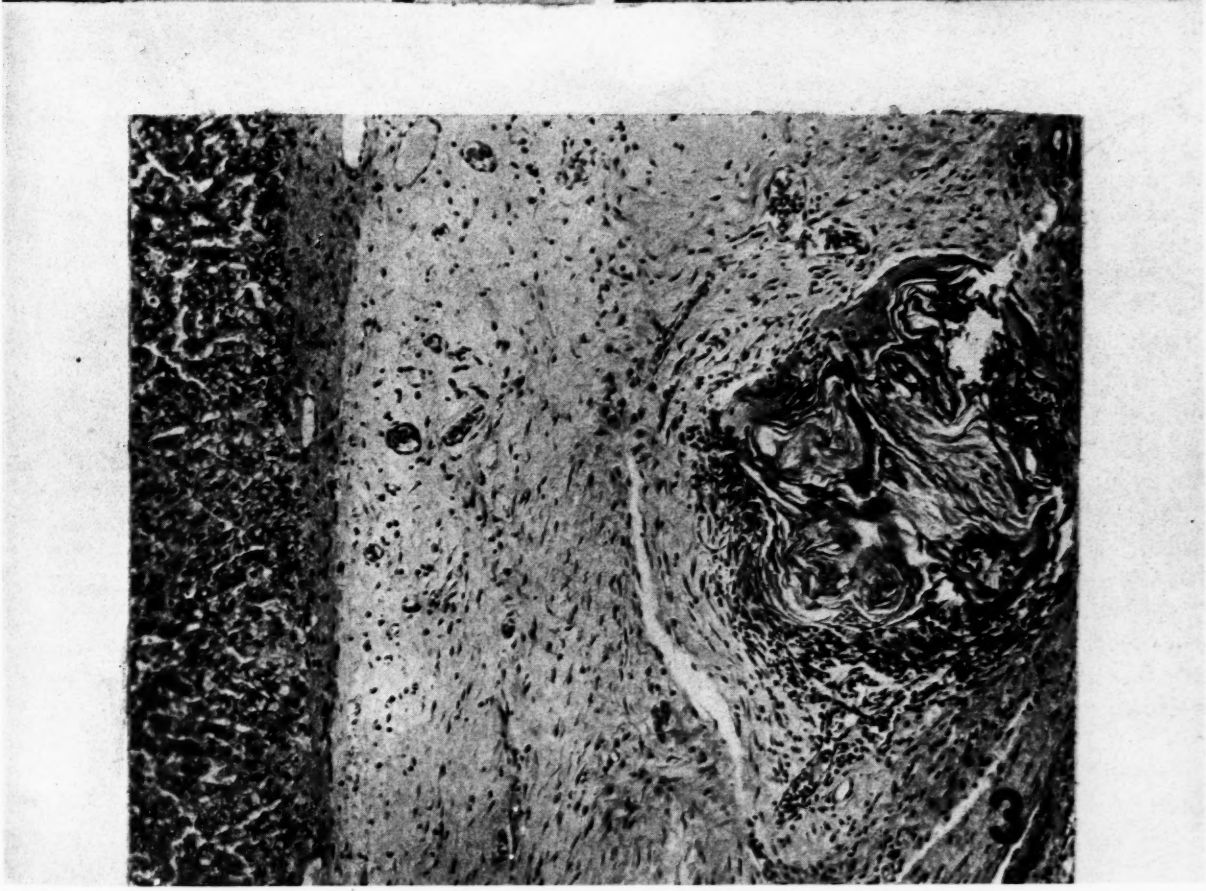
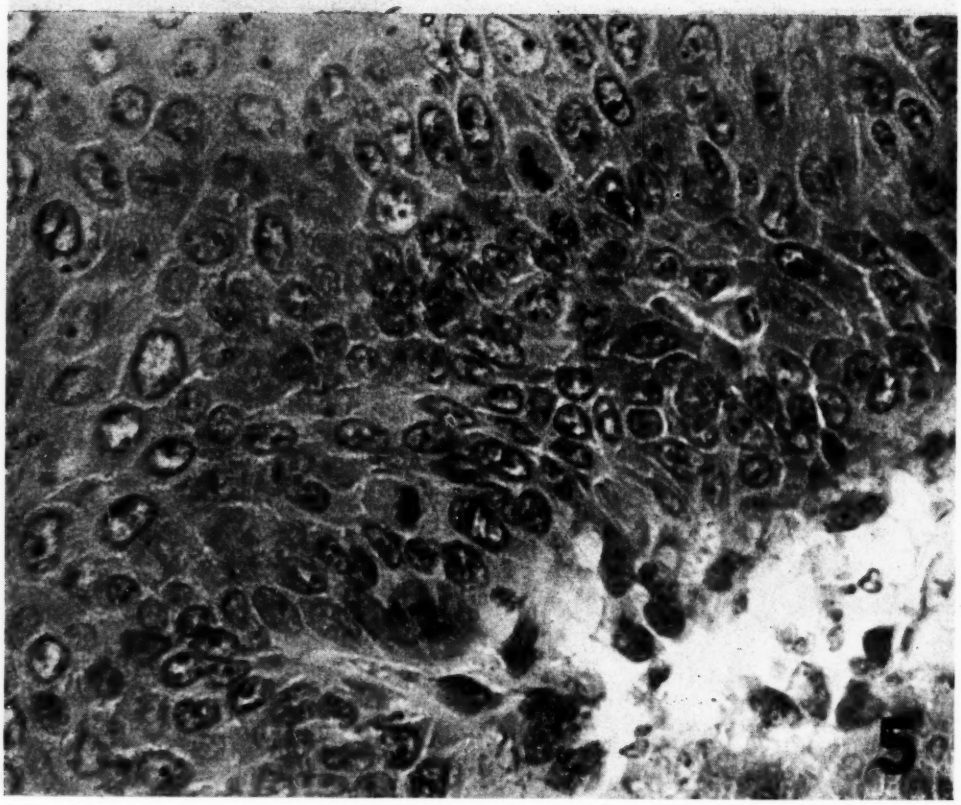


FIG. 1.—A small sheet of epithelial cells in a tissue culture of rabbit skin. This culture was not treated with papilloma virus. The dark mass on the left is the original explant; the group of paler cells near the center of the figure is the outgrowth. This culture was five days old. The photomicrograph was taken through the wall of the culture tube and the cells were not fixed or stained. Mag. × 225.

FIG. 2.—A large sheet of epithelial cells in a tissue culture of rabbit skin to which was added papilloma virus. The picture was taken on the fourth day of culture. Mag. × 110.

FIG. 3.—Fibrosis surrounding a degenerating clump of squamous epithelium in the liver. The skin, in this case, had been removed from the same rabbit into which it was later implanted. Meanwhile it had been grown in tissue culture without the addition of papilloma virus. Mag. × 72.



FIGS. 4-5

had been obtained originally, as well as in another rabbit. Six pairs of rabbits were so treated. Another group of 6 pairs of rabbits served as controls. Cultures of skin not treated with virus were planted in the rabbit from which the skin had been removed, and also in another rabbit. Also, virus alone was injected into the livers of 6 domestic rabbits.

The implants were made by exposing the liver of the anesthetized rabbit through an abdominal incision. Small slits were made in the liver with the point of a scalpel, and the tissue culture explants were tucked into these slits with a pair of forceps. The incision was then closed by suture, and the rabbits were kept for from 3 to 8 weeks, after which they were sacrificed and the implantation sites examined.

In rabbits in which virus alone had been injected into the liver no lesions of any kind were found, nor were any expected, since it is well established (7) that the papilloma virus is specific for the skin epithelium of the rabbit.

Rabbits bearing implants of skin which originally came from other rabbits, whether the culture of the skin had been treated with virus or not, showed no tumors. The implanted fragments in some cases had persisted as small white flecks surrounded with scar tissue, and microscopic sections (Fig. 3) revealed fibrosis and an occasional nest of degenerating epithelial cells.

In contrast, rabbits bearing implants of their own skin which had been treated with papilloma virus showed regularly, opaque, white, solid nodules of tumor tissue 2.5 to 3 cm. in diameter, at the sites of implantation. These nodules were many times the size of the original explants (Fig. 4). Microscopic sections of these nodules revealed masses of proliferating squamous epithelium containing many mitotic figures (Fig. 5). As shown in the photomicrographs (Figs. 6-7), the proliferating epithelium formed a papillomatous pattern even when growing in the liver. These growths were similar to those obtained when papillomas from the skin are transplanted into the liver of the same rabbit (7).

DISCUSSION

The foregoing experiments indicate that the rabbit papilloma virus is capable of inducing neoplasia *in vitro*. The only other experiments in which neoplasms were produced by a virus *in vitro* are those of Carrel (1) and Carrel and Ebeling (2), using the Rous sarcoma. They concluded that the virus was present in macrophages, which they thought to be the essential cells constituting the Rous sarcoma.

When, in the present experiments, tissue cultures of rabbit skin were treated with papilloma virus and later implanted in rabbit livers, tumors resulted only when the skin was implanted in the rabbit from which it had been removed originally. This indicates that the skin had not lost its individual specificity during tissue culture and is also in agreement with the observation that the tissue cells of the rabbit papilloma cannot be successfully transplanted from one rabbit to another, whereas they can be transplanted into different situations in the rabbit in which they are growing (8).

It would be of interest to determine whether or not malignant growth can be induced by the papilloma virus *in vitro*. Malignant change of the papilloma occurs in the domestic rabbit in a considerable proportion of cases when it is allowed to grow for a sufficient period (9). To determine the occurrence of malignancy *in vitro* one might observe the behavior of long-term tissue cultures subsequently implanted in rabbit livers, using invasive growth and metastases as indication of malignancy. Also one might use heterologous intra-ocular transplants (6) as a test for malignancy. Heterologous transplantations are successful only with malignant tumors, so that the benign papilloma should fail to grow in the eye of an alien species, whereas the squamous cell carcinoma should grow.

Other problems might well be explored by using the papilloma virus *in vitro*. For example, since the domestic rabbit bearing papillomas is highly resistant to further treatment with the virus (10), would skin removed from such a rabbit and grown in tissue culture remain resistant to the virus? As it is difficult to recover the virus from the papillomas of domestic rabbits (10), would it be possible to recover it from tissue cultures of domestic rabbit skin?

The use of papilloma virus and rabbit skin *in vitro* should serve as an excellent method for investigations of virus-cell relationships.

DESCRIPTION OF FIGURES 4 AND 5

FIG. 4.—Nodules of tumor in the liver of a rabbit. These nodules developed from implanted tissue culture fragments of rabbit skin which had been treated with papilloma virus. The implants were made into the liver of the same rabbit from which the skin had been taken. Three weeks after implantation, the fragments have grown to many times the size of the original implants.

FIG. 5.—A photomicrograph of a section from one of the tumor nodules illustrated in Fig. 4, showing masses of squamous epithelium which forms the bulk of the growth. Two mitotic figures are apparent. H. & E. stain. Mag. × 280.

FIGS. 6 and 7.—These two photomicrographs show the papillomatous pattern characteristic of such tumor nodules as illustrated in Fig. 4. Masses of keratinized epithelium form the central parts of these tumors. The more actively proliferating cells are at the periphery. Fig. 6, Mag. × 17. H. & E. stain. Fig. 7, Mag. × 80.

SUMMARY AND CONCLUSIONS

1. Experiments were designed to determine whether neoplasia can be induced in tissue cultures of rabbit skin by papilloma virus.

2. The criteria adopted for induction of neoplasia were:

a. Increased growth activity of epithelial cells in the cultures after introducing papilloma virus.

b. Formation of relatively large tumors in the liver of the rabbit following implantation of such tissue cultures.

3. Both criteria were met by the experiments and it is therefore concluded that papilloma virus is capable of inducing neoplasia *in vitro,* namely in tissue cultures of rabbit skin.

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Effect of Progesterone on the Development of Mammary Cancer in C3H Mice

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In experiments by Lipschütz, Murillo, and Vargas (3) it was shown that progesterone given in a proportion of 150:1 of estradiol prevented the uterine fibromatosis otherwise provoked by estradiol in guinea pigs. Heiman (1) investigated the effect of progesterone on the incidence of mammary cancer in RIII mice and found that the occurrence of these tumors was considerably reduced thereby. Progesterone counteracted also the growth-accelerating action of the estrogen on the mammary glands.

Furthermore, progesterone and testosterone have an affinity in some of their biological activities, and Lipschütz, Vargas, and Ruz (4) found that, like progesterone, testosterone propionate supplied in a proportion of 22:1 prevented the induction of uterine fibromas by estradiol. Lacassagne (2), and Nathanson and Andervont (5) reported that spontaneous mammary cancer in mice could be prevented to a large extent by the repeated administration of testosterone propionate. Moreover, it has been repeatedly suggested that in addition to hereditary factors in pure strain mice with high incidence of mammary cancer an excessive secretion of estrogen may determine the incidence of these tumors, and that this excess may be held in check by testosterone and progesterone. In view of these varied considerations it seemed of interest to investigate the effect of progesterone on the incidence of spontaneous mammary tumors in a pure strain of mice.

Method of the Experiment and Results

Female mice of C3H strain were used. All the mice were kept in the same room, from 1 to 5 mice in one metal cage. The diet consisted of Millard-Marsh basal diet, oats, puppy biscuit, rat cake, brown bread, marmite, cod liver oil, and water *ad libitum*. The treatment started after one litter was born; and the mothers were permitted to suckle the young.

Thirty animals received subcutaneous injections each week of 1 mgm. crystallized progesterone dissolved in 0.2 cc. arachis oil; 20 controls received the oil only. The mice were inspected weekly for appearance of tumors.

Tables I and II show that no difference could be established in the incidence of tumors, nor in the age of the appearance of the tumors in the treated and untreated mice. The treated mice received 6 to 34 mgm., in the average 21 mgm., progesterone. Up to date, 3 animals of each group remained free of tumors. The tumor-free mice were not invariably daughters of tumor-free mothers.

Table I: Frequency and Age of Incidence of Tumors in Progesterone-Treated Animals

Tumor in mother of animal in experiment	Age of mice in days		Number of injections
	Treatment started	Tumor appeared	
+	73	335	29
+	92	253	19
+	35	302	34
+	35	302	34
—	220	469	34
+	212	419	23
+	223	473	32
+	66	302	28
+	69	275	23
+	66	245	20
—	190	233	6
+	84	nil(340)*	32
+	102	234	16
—	94	275	22
+	81	nil(326)*	30
+	87	220	16
+	76	258	24
+	115	262	16
+	75	229	19
—	133	252	12
+	82	180	14
+	147	245	13
+	126	231	13
+	167	258	11
+	140	214	10
+	110	215	15
+	131	265	18
+	95	nil(229)*	18
Mean	116	280	20.8

(3 animals without tumors)

* The figures in brackets represent the age of the animal at the end of the experiment.

Note: Two of the treated and 1 control animal died (without tumor) during the first 3 to 4 weeks of the experiment and are not included in the tables.

TABLE II. FREQUENCY AND AGE OF INCIDENCE OF TUMORS IN CONTROL ANIMALS

Tumor in mother of animal in experiment	Age of mice in days		Number of injections
	Treatment started	Tumor appeared	
+	73	344	32
—	129	349	26
+	143	210	8
+	157	245	10
+	178	335	19
+	178	224	5
—	224	453	27
+	145	338	25
+	97	255	18
+	185	259	10
+	97	227	17
+	88	290	23
+	88	340	32
+	78	315	21
+	196	237	5
—	91	nil(325)*	31
+	63	nil(274)*	28
—	119	196	10
—	69	nil(273)*	27
Mean	126	288	19.8

(3 animals without tumors)

* The figures in brackets represent the age of the animal at the end of the experiment.

DISCUSSION

While the present investigations were in progress the paper by Heiman (1) appeared dealing with the effect of progesterone and testosterone propionate on the mammary tumors in RIII mice. The complete failure in the present experiments to produce any beneficial effect by the administration of progesterone on the mammary cancer in mice may be attributable to the employment of a different strain of mice. The dosage of progesterone was higher than in the experiments of Heiman which makes a direct comparison difficult. On the supposition that progesterone acts by reducing the output of gonadotropic hormone of the pituitary it seems unlikely that an increased dose would counteract this effect. The different results observed show the need for care in attempting to generalize results even in different strains of the same species and emphasizes the difficulty of attempting to carry over results obtained in experimental animals to human pathology..

SUMMARY

Thirty mice of a high mammary cancer strain (C3H) received subcutaneous injections of 1 mgm. of progesterone weekly. No differences could be established in the frequency and the age of the appearance of tumors in these mice and in the 20 control animals receiving injections of the solvent only.

ACKNOWLEDGMENTS

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The Influence of Sex Hormones upon the Hepatic Lesions Produced by 2-Acetaminofluorene*

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The carcinogenic action of 2-acetaminofluorene has been described by Wilson, De Eds and Cox (14) and by Bielschowsky (1). This compound is particularly interesting because of the fact that, when fed to stock albino rats (14) and to descendants of Wistar rats (1), it produced a greater variety of tumors than most known carcinogens. Bielschowsky (2) reported the development of benign and malignant tumors of the thyroid in rats following simultaneous administration of 2-acetaminofluorene and the antithyroid drug, allylthiourea, the carcinogen alone being without influence upon the normal gland. Adenomas of the thyroid, but not malignant tumors, have been observed in rats fed allylthiourea (3), brassica seed (5) and thiouracil (10) for long periods of time.

This observation of the susceptibility of the stimulated goitrous thyroid to the tumor-producing action of the carcinogen suggested that it might be interesting to investigate the influence in this connection of other agents that stimulate growth of specific tissues. Androgenic and estrogenic hormones were chosen for this purpose.

METHODS

Two hundred and three rats of the Sherman strain weighing about 75 gm. each were divided into 6 experimental groups. All received 0.03 per cent 2-acetaminofluorene in the following diet: corn meal, casein, alfalfa, linseed oil, bone ash, and NaCl, with supplements of brewers' yeast and cod liver oil.

Group 1 (*Control*): 80 rats received no additional treatment.

Group 2 (*Thiouracil*): 30 rats received 0.05 per cent thiouracil in the drinking water.

Group 3 (*Exogenous estrogen*): 36 female rats received 0.125 mgm. of estradiol dipropionate (in sesame oil) intramuscularly 3 times weekly.

Group 4 (*Endogenous estrogen*): 20 female rats received 25 I.U. of pregnant mare serum (PMS) gonadotrophin (in aqueous solution) intramuscularly 3 times weekly.

Group 5 (*Exogenous androgen*): 20 male rats received 0.5 mgm. of testosterone propionate (in sesame oil) intramuscularly 3 times weekly.

Group 6 (*Endogenous androgen*): 17 male rats received 25 I.U. chorionic gonadotrophin (in aqueous solution) intramuscularly 3 times weekly.

Treatment was continued in all instances until the time of death or sacrifice.

Tissues were fixed in Bouin's fluid and slides were stained with hematoxylin and eosin. Other slides were stained with Masson's trichrome stain for connective tissue, Marchi's fluid for fat, and Best's carmine for glycogen (absolute alcohol fixation).

RESULTS

It became obvious early in the course of the experiment that the hepatic lesions in the animals receiving sex hormones differed strikingly from those in the control (carcinogen only) and thiouracil-treated groups. This communication is concerned particularly with the 76 animals that have been examined after 44 to 316 days of treatment.

Mammary carcinoma occurred in 3 female controls. Of the thiouracil group, 7 animals developed tumors of the thyroid, 1 with pulmonary metastases, and another a carcinoma of the uterine horn. A squamous cell papilloma occurred in the bladder of 1 testosterone-treated rat.

All animals receiving thiouracil exhibited pronounced hyperplasia and hypertrophy of the thyroid. The majority of those receiving estradiol developed pyometra and one receiving PMS gonadotrophin had a pyosalpinx. The target organ response to testosterone was less marked than is usually observed in animals receiving this substance alone and the target organs in the PMS and chorionic gonadotrophin-treated animals showed no evidence of stimulation.

The hepatic lesions have been classified as (a) cystic and (b) neoplastic (hepatoma; cholangioma). The former were regarded as benign and the latter as malignant, solely on the basis of histologic characteristics, except in 3 instances in which there were pulmonary metastases. The incidence of these lesions in the various experimental groups is presented in Table I.

Controls (*carcinogen only; 21 animals; 18 females, 3 males*).—After 185 to 316 days of treatment, cystic lesions were observed in 15 (71 per cent) and neoplasms in 6 instances (29 per cent), the latter occurring in 2 of the 3 males and 4 of the 18 females of this

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group. No hepatic neoplasms were seen in animals treated for less than 180 days.

Thiouracil (15 animals; 9 females, 6 males).—After 44 to 316 days of treatment, cystic lesions were observed in 3 instances (20 per cent) at 130, 185 and 316 days, respectively. There were no hepatic neoplasms in this group and no sex difference was apparent.

Estradiol (13 animals).—After 61 to 256 days of treatment, cystic lesions were observed in 12 (92 per cent) and neoplasms in 9 (60 per cent) of instances; the earliest cysts occurred at 132 days and the earliest neoplasm at 165 days.

PMS gonadotrophin (endogenous estrogen; 12 animals).—After 185 to 300 days of treatment, cystic lesions were observed in 12 (100 per cent) and hepatic neoplasms in 4 (33 per cent) of the animals. One had pulmonary metastases. The earliest hepatoma occurred at 201 days.

Testosterone (10 animals).—After 206 to 302 days of treatment, cystic lesions were observed in 10 (100 per cent) and hepatic neoplasms in 8 (80 per cent) of instances. The earliest hepatoma occurred at 206 days.

Chorionic gonadotrophin (endogenous androgen; 5 animals).—After 160 to 250 days of treatment, cystic and neoplastic lesions were observed in all animals.

Table I: Incidence of Cystic and Neoplastic Lesions in Liver

Group	No. of animals	Cysts					Neoplasms			
		Slight	Moderate	Pronounced	Total	%	Cholangioma	Hepatoma	Total animals	%
Control	21	9	6	—	15	71	1	6	6	29
Thiouracil	15	3	—	—	3	20	—	—	0	0
Estradiol	13	—	4	8	12	92	4	8	9	69
PMS Gonadotrophin	12	3	5	4	12	100	—	4	4	33
Testosterone	10	1	5	3	10	100	2	7	8	80
Chorionic Gonadcotrophin	5	—	3	2	5	100	1	5	5	100

The significant histologic changes could be classified as (a) degenerative and regenerative, (b) cystic and (c) neoplastic.

Degenerative and regenerative changes.—There occurred in some degree in all groups of animals, being generally minimal in those receiving thiouracil, slight in the control group (carcinogen only) and most pronounced in those receiving testosterone. Even in the latter, these changes were seldom advanced.

The characteristic features of these lesions were as follows: The hepatic cells were swollen, with granular or vacuolated cytoplasm and well stained nuclei. The sinusoids were compressed by the swollen cells, but in early cases the normal lobular architecture was well preserved. In animals treated for longer periods the architecture became somewhat distorted, with variation in size and shape of the lobules, irregularity in position of blood vessels and variation in size and staining reaction of cells.

Proliferation of young, cellular, connective tissue was observed in all groups, paralleling the changes noted above; in no instance was it striking, the Masson stain failing to reveal a sharp increase in collagenous fibers.

Regenerative phenomena, which paralleled the degenerative and proliferative changes, were represented by large hepatic cells with conspicuous nuclei and by increased mitotic activity, occurring particularly in the lobules exhibiting architectural distortion.

Cystic changes.—Both in the gross and microscopically, the most striking and most constant changes in the liver were cystic in nature. The cysts varied widely in size, shape and number and usually occurred in groups; the individual members of these groups were often partially separated by broken-down septa, suggesting that large cysts were formed by fusion of smaller ones. At autopsy, they contained clear fluid; in stained sections they were at times empty and occasionally contained a granular, pink-staining substance, or mononuclear cells. The smaller cysts were lined by cuboidal epithelium and the larger by flat epithelial cells. They were smallest and least numerous in the thiouracil-treated animals and most conspicuous in those receiving estradiol, in which they usually occurred as numerous collections of large cysts of a honey-combed appearance (Fig. 3).

Occasionally cysts were encountered, lined by tall columnar cells with a rather acidophilic cytoplasm, frequently forming papillary projections into the lumen (Fig. 4). This lesion conformed to that designated as "cystadenoma" by Opie (8), and occurred most frequently in the animals receiving chorionic gonadotrophin, rarely in the controls and never in the thiouracil group.

Neoplastic changes.—The neoplastic lesions were similar to those described by Orr (9), Edwards and White (4) and Opie (8) in rats treated with *p*-dimethylaminoazobenzene. They were classified as (a) hepatomas and (b) cholangiomas, on morphologic grounds. Both occurred in multiple foci throughout the liver.

The hepatomas were of two varieties conforming to those designated by Opie as "adenohepatoma" and "trabecular hepatoma," with both types often repre-

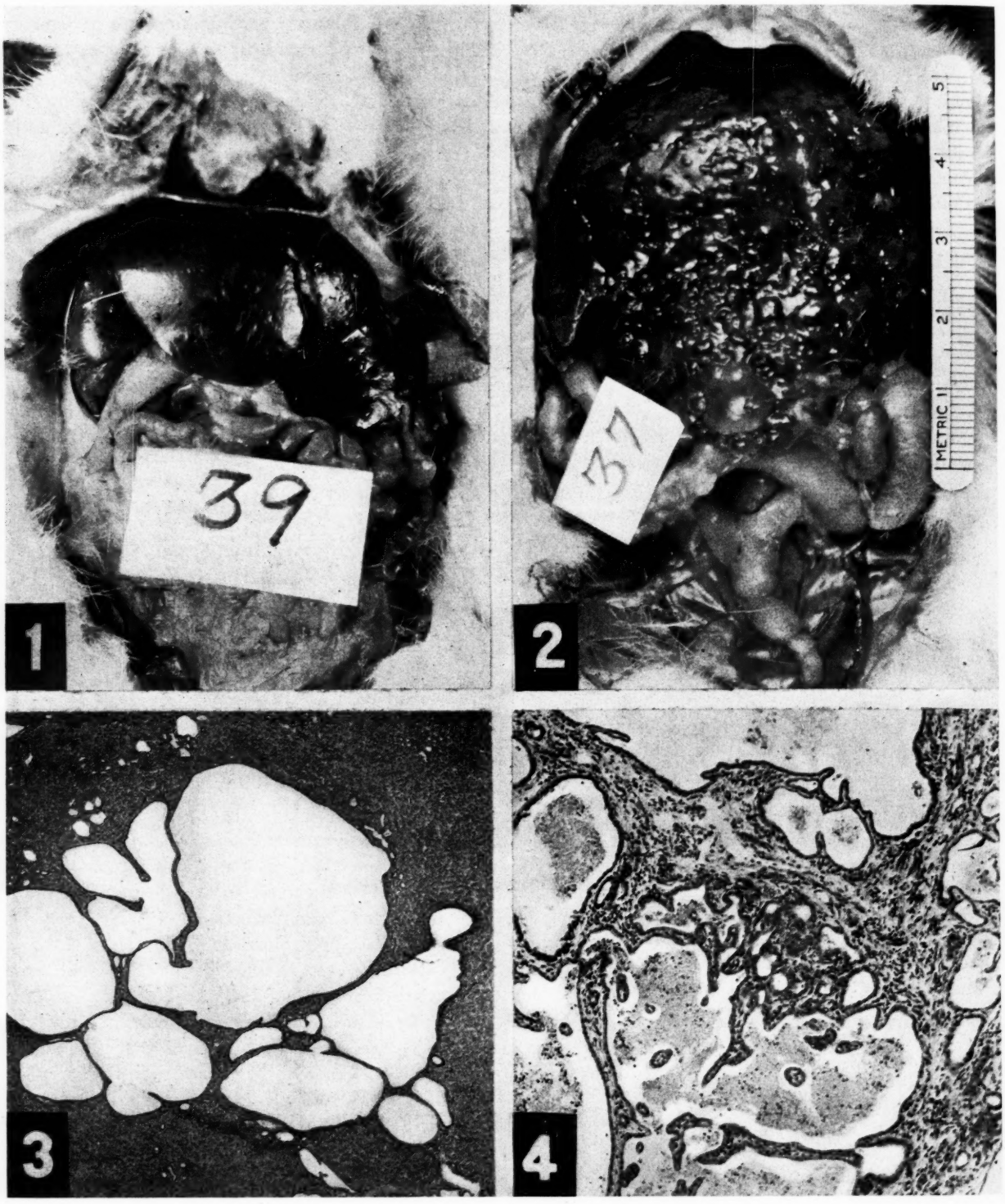


FIG. 1.—Liver of control rat (carcinogen alone) treated for 258 days.

FIG. 2.—Liver of rat treated with estradiol for 217 days. Note pronounced cystic changes.

FIG. 3.—Liver of rat treated with estradiol for 217 days, showing pronounced cystic lesions. Mag. × 30.

FIG. 4.—Liver of rat treated with chorionic gonadotrophin 250 days, showing epithelial proliferation into lumen of cyst, constituting a cystadenomatous lesion. Mag. × 60.

sented in the same nodule (Fig. 9). The constituent cells were large and palely acidophilic, resembling liver cells, with vesicular nuclei and distinct nucleoli. In the former the cells were arranged in acinar formation, the acini being surrounded by dilated sinusoids lined by endothelial cells (Fig. 5). These adenomatous areas gradually merged into relatively normal liver tissue. In the other variety the cells, which were often large and polyhedral, frequently with conspicuously irregular-shaped nuclei, exhibited no regularity in their arrangement. They were frequently fused together or formed giant cells, with multiple or lobulated, deeply-staining nuclei; many mitotic forms were present (Figs. 6 to 8); the cell outlines were often indistinct and irregular, the neoplastic cells frequently infiltrating between the cells of otherwise normal liver cords. Areas of hemorrhage and cystic change were commonly observed adjacent to the hepatomatous nodules. Both varieties of hepatoma occurred most frequently in animals treated with testosterone and chorionic gonadotrophin but were also common in those receiving estradiol. True hepatomas were differentiated from nodules of regenerating liver cells by the preservation in the latter of the normal, fundamental relationship between liver cells and sinusoids and by distortion of this relationship in the former, with marked irregularity in size and arrangement of the cells and in the staining reactions and appearance of their nuclei. Glycogen was present in the cells of the non-neoplastic areas of the hepatic parenchyma, although frequently in somewhat irregular distribution, but very few of the neoplastic cells contained glycogen granules. Multiple pulmonary metastases from these tumors were noted in 2 animals (Figs. 10 to 14).

The cholangiomas (Figs. 11 to 13) consisted of columnar cells, with a basophilic cytoplasm and oval nucleus, exhibiting a tendency to form acini and tubules. When arranged circularly, they resembled regenerating bile ducts. These gland-like structures were usually irregularly arranged, lined by one or several layers of tall, basophilic cells and separated by a cellular stroma containing lymphocytes, monocytes, and fibrocytes. There were many mitotic forms. This lesion closely resembled adenocarcinoma (Fig. 13).

DISCUSSION

These observations indicate that exposure to increased quantities of exogenous or endogenous estrogen or androgen hastens the development of and intensifies the hepatic lesions induced by 2-acetaminofluorene in the rat (Sherman). The lesions seem identical with those produced by *p*-dimethylaminoazobenzene. As noted by Opie in the case of the latter, both cystic and neoplastic lesions occurred frequently in the absence of significant cirrhosis or extensive hepatocellular damage. It is improbable, therefore, that this effect of the sex hormones is dependent fundamentally upon a hepatotoxic action, which has been attributed to estrogens by some (7, 13) and denied by others (11, 12). Moreover, no hepatotoxic effect has been reported for androgens or gonadotrophins.

Because of the absence at the time of sacrifice of evidence of significant stimulation of the ovaries and testes in the gonadotrophin-treated animals, their exposure to increased quantities of estrogen or androgen at some time during the experimental period must remain, for the present, an assumption. This seems justified, however, because of the dosage employed, and because of the similarity between the hepatic lesions in the animals receiving gonadotrophins and exogenous estrogen and androgen. One would otherwise have to assume a direct effect of gonadotrophin upon the liver identical to that of estradiol and testosterone, which is highly improbable. The absence of target organ response may be due to the development of antigonadotrophins.

The difference in response of the control, thiouracil-treated and sex hormone-treated animals does not appear to be due to variations in intake of food or carcinogen. In general, appetite was diminished in animals receiving thiouracil and especially in the estrogen-treated group, the intake of acetaminofluorene being correspondingly lowered. However, the fact that an adequate quantity of carcinogen was taken by both of these groups was evidenced by the consistent urinary excretion of a substance that stained the fur and pine shavings bright orange (14) and by the development of neoplasms, in the thyroid and uterus in the thiouracil series and in the liver in a large proportion of the estrogen series. Moreover, the food intake remained normal in the groups receiving testosterone and gonadotrophins during the greater part of the experimental period, as indicated by gain in weight. It has been noted previously, that the occurrence of the cystic and neoplastic lesions was not paralleled by degenerative or fibrotic processes in the liver, as might result from dietary causes such as increased cystine and decreased choline or methionine intake.

The accelerated development of these lesions under the influence of estrogen and androgen is particularly interesting because of the role of the liver cell in the intermediary metabolism and excretion of these hormones and related steroids. Any attempt to explain the phenomenon on this basis would be purely speculative, but the implications are obvious. The sex hormones or their metabolites may act as cocarcinogens, or carcinogens may be formed from them as a result of perversion of their metabolism by the hepatic cells under the influence of aminofluorene. The effect of estrogen in males and androgen in females is being investigated.

Our findings differ in certain respects from those

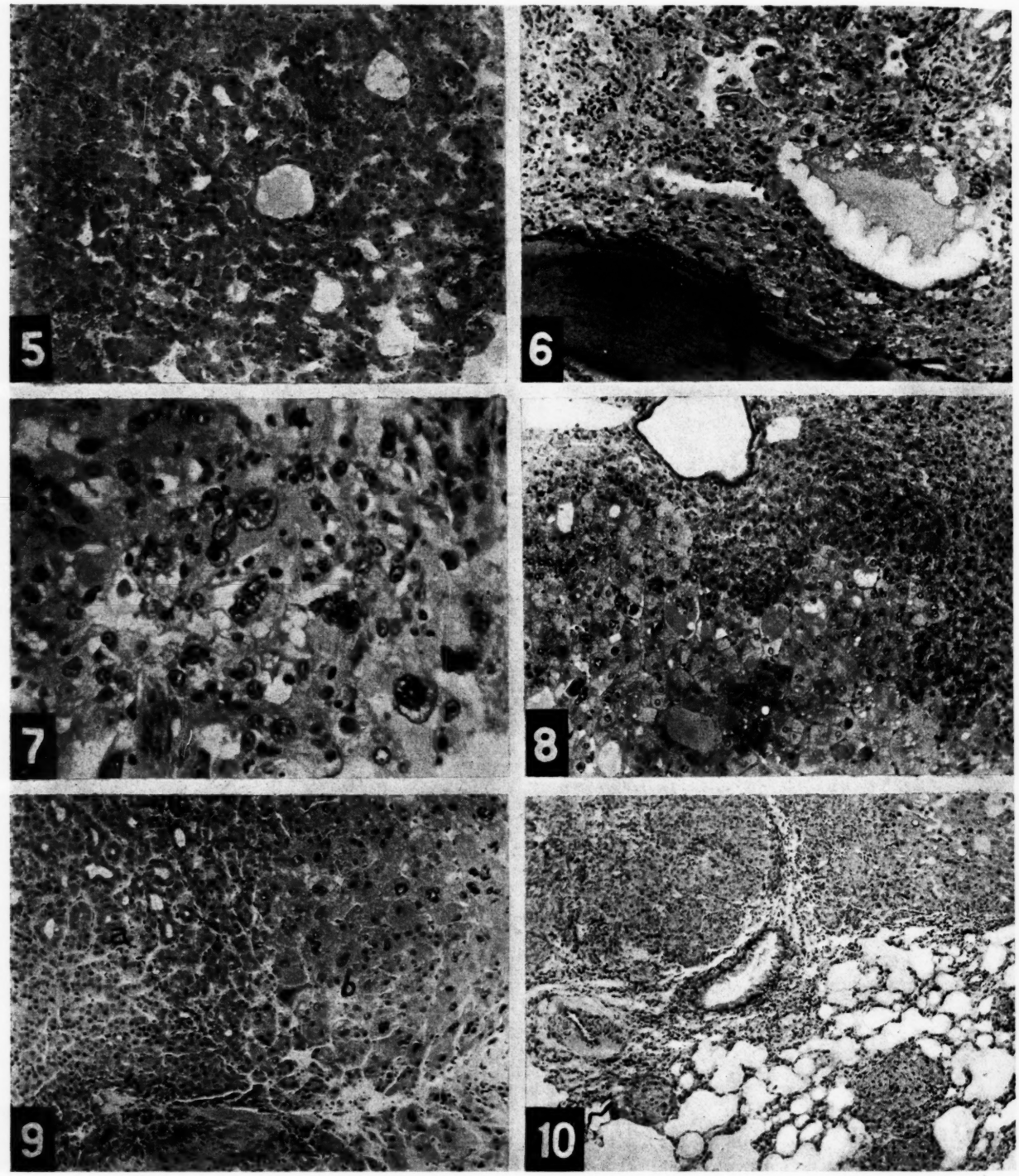


FIG. 5.—Liver of rat treated with PMS gonadotrophin for 195 days, showing a hepatoma, with adenomatous arrangement of proliferating cells, with dilated sinusoids and cysts. Mag. × 110.

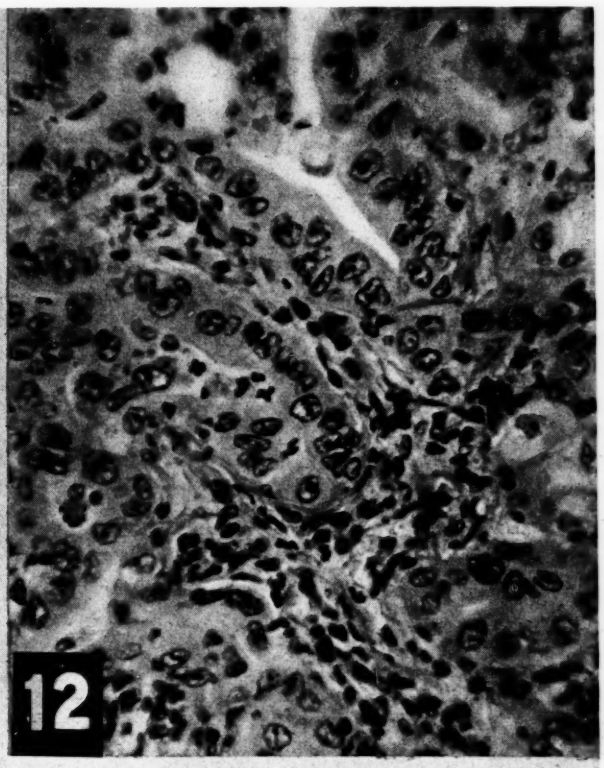
FIG. 6.—Liver of rat treated with PMS gonadotrophin for 162 days. Hepatoma, with marked variation in size and shape of cells and area of hemorrhage at corner. Mag. × 86.

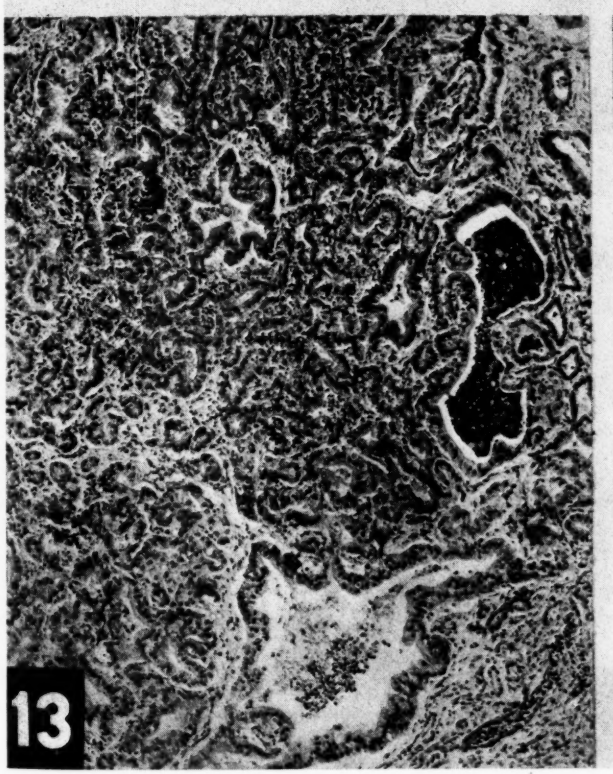
FIG. 7.—Higher magnification of Fig. 6, hepatoma, showing the syncytial, giant neoplastic cells with bizarre, hyperchromatic nuclei. Mag. × 312.

FIG. 8.—Liver of rat treated with estradiol for 165 days, showing margin of hepatoma infiltrating adjacent liver tissue. Mag. × 86.

FIG. 9.—Liver of rat treated with testosterone for 252 days. Hepatoma, exhibiting both adenomatous (a) and trabecular (b) arrangements in the same nodule. Mag. × 86.

FIG. 10.—Metastatic lesion in lung from a hepatoma in a rat treated with testosterone for 297 days. Mag. × 55.





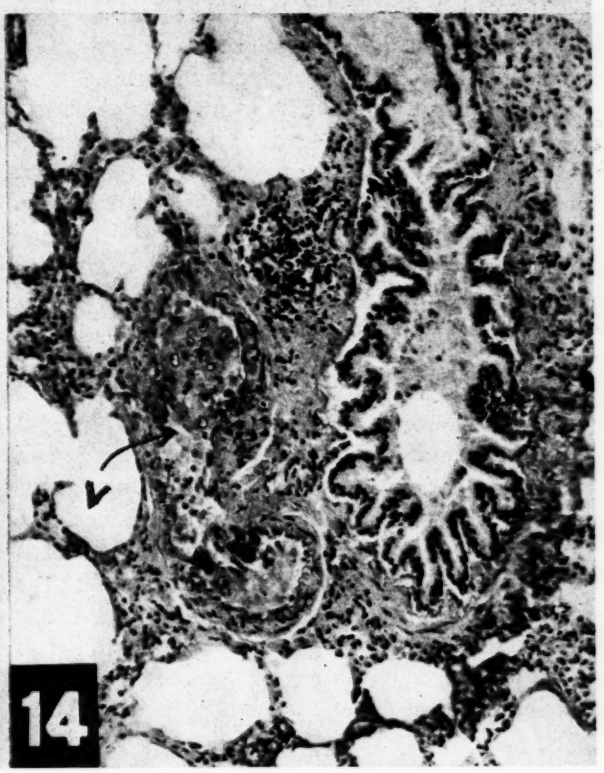


FIG. 11.—Liver of rat treated with estradiol for 176 days, showing cholangioma with irregular gland and cyst formation. Mag. × 110.

FIG. 12.—Liver of rat treated with estradiol for 289 days. Tall columnar cells of cholangioma in tubular or acinar arrangement. Mag. × 400.

FIG. 13.—Liver of rat treated with chorionic gonadotrophin for 250 days, illustrating close resemblance of cholangioma to adenocarcinoma. Mag. × 65.

FIG. 14.—Lung of rat treated with PMS gonadotrophin for 278 days, showing metastatic hepatoma cells in bronchial blood vessel (V). Mag. × 150.

reported previously. Wilson, DeEds and Cox (14) found malignant tumors in 19 of 39 (48.7 per cent) and Bielschowsky (1) in 93 of 104 rats (89.4 per cent), as compared with only 7 in our control group of 21 animals (33.3 per cent). Hepatic neoplasms were observed by Bielschowsky in 59 per cent of cases (83 per cent males; 30 per cent females; 100 per cent castrate males; 45 per cent castrate females) and in only 29 per cent of our controls. This discrepancy may be due in part to the difference in strain. It may also be due to the fact that the basic diet in the present experiment, which was the same as that employed by Wilson, DeEds and Cox (14), was supplemented by brewers' yeast. This may have made the liver more resistant to the effect of the carcinogen, but it is difficult to understand how it could have affected other tissues in this manner.

The extremely low incidence of degenerative, fibrotic and cystic changes and the absence of neoplasms in the livers of the thiouracil-treated animals was most striking. As indicated above, it does not seem to be due to dietary factors or to inadequate intake of the carcinogen. No such protective action was apparent in Wistar rats receiving allylthiourea (2), 50 per cent of which developed hepatomas. It is of interest in this connection that György and Goldblatt (6) found that thiouracil exerts a preventive effect on the production of dietary cirrhosis in rats. They suggested that this phenomenon may be due to decrease in the requirement for methionine incident to the lowered metabolic rate. Further observations will be reported elsewhere.

The original purpose of this study was to determine whether hyperplasia of the uterus, ovaries, prostate, seminal vesicles, and testes, induced during a period of exposure to acetaminofluorene, would lead to the development of neoplasms in these organs, as in the case of the hyperplastic thyroid. We have observed thyroid adenomas following prolonged treatment (360 days) with thiouracil alone. Simultaneous administration of acetaminofluorene may merely accelerate this process. It is of interest, however, and possibly of significance, that the hyperplasia of target organs of the sex hormones is a functioning hyperplasia, whereas that induced in the thyroid by goitrogenic agents is non-functioning. This fundamental difference merits serious consideration as a possible determining factor in the relationship between simple and neoplastic growth of tissue.

SUMMARY

Excessive quantities of endogenous or exogenous estrogen and androgen accelerated and intensified the development of cystic and neoplastic hepatic lesions induced in Sherman rats by 2-acetaminofluorene. This phenomenon may be related to the role of the liver in the intermediary metabolism and excretion of the sex steroids.

Thiouracil appeared to exert a strikingly protective action upon the liver as regards the development of both cystic and neoplastic lesions.

No tumors occurred in the hyperplastic target organs of the sex hormones, in sharp contrast to the high incidence of tumors of the thyroid in rats receiving thiouracil simultaneously with the carcinogen. The fact that the hyperplasia induced by the sex hormones is a functioning hyperplasia, whereas that induced in the thyroid by thiouracil is functionless, may possibly be a determining factor in the relationship between simple and neoplastic growth of tissue.

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Tumors of the Salivary and Parathyroid Glands in Rats Fed with 2-Acetylaminofluorene

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Wilson (3) and his associates induced tumors in rats fed 2-acetylaminofluorene in a prepared diet. The tumors arose in many organs but none appeared in the salivary glands. Bielchowsky (1) reported similar findings with this compound and recorded tumors of the thyroid gland, also, after thiouracil had been added to the diet (2). No changes of any sort in the parathyroid glands were reported.

In our experiments, 36 female and 23 male Wistar rats 2½ to 3 months old were given an oily mixture (peanut oil) of 2-acetylaminofluorene with a syringe bearing a curved needle for introduction into the pharynx. Of this mixture, 1 cc. containing 10 mgm. of the drug was administered every other day, and later 20 mgm. were given every third day. The total amount given ranged between 250 and 650 mgm. during 111 to 227 days. The animals were fed on one part Purina dog chow checkers and two parts Rockland rat diet, allowed water *ad libitum*, and observed until death.

Twenty-two of the treated rats developed nodular swellings in one or both of the submaxillary regions. These swellings extended to the subcutaneous areas of the chest and axillae. Autopsy revealed enlarged submaxillary glands and many small discrete or large confluent cysts containing a yellowish oily fluid (peanut oil). The cystic nodular masses ranged in size between $1.5 \times 1.6 \times 2.4$ cm. and $3.0 \times 2.5 \times 2.2$ cm., and displaced or covered the submaxillary glands. By further dissection the swellings were traced to the mediastinum. Several animals showed white exudates on the pleura and pericardium, and effusions were noted in the pleural and pericardial spaces. The changes in the liver varied between passive congestion, cirrhosis, cysts, and adenoma. The adrenal glands and the thyroid also contained cysts, which, however, were not filled with yellow oily fluid. Four of the treated females, one having been fed 435 mgm. of 2-acetylaminofluorene in 213 days, had litters.

In addition to cystic swellings, 12 animals developed tumors, as follows:

Tumor	No. of animals
Adenocarcinoma of submaxillary gland	2
Adenoma of submaxillary gland	3
Mammary adenocarcinoma	2
Parathyroid adenoma	2
Adenoma of liver	1
Sarcoma of neck	1
Thyroid adenoma	1

Only neoplasms of the submaxillary and parathyroid glands not hitherto reported will be described. One tumor of the left submaxillary gland appeared in a female rat that had received 340 mgm. of 2-acetylaminofluorene in 4 months. Eleven weeks after the last dose of the drug the animal was sacrificed. The tumor, soft, purple, nodular, and not encapsulated, measured $2.8 \times 2.6 \times 1.5$ cm. Microscopic section (Fig. 1) showed a cystic tumor adjoining normal salivary glands. This adenocarcinoma had distorted architecture which included large cysts containing papillary projections of epithelium. Many of the cystic spaces were completely filled with masses of epithelium. Fig. 2 shows invasion of the subcutaneous connective tissue by groups of cancer cells. The cells were irregularly cuboidal and the cytoplasm contained mucous secretion demonstrated by the mucicarmine stain. Numerous mitoses were evident.

The adenomas of the submaxillary gland appeared as isolated areas of hyperplastic ducts and acini, in which the normal cuboidal epithelium had been replaced by columnar epithelium (Fig. 3). These localized adenomas showed a transition from normal tissue to the adenocarcinoma previously described.

Serial sections of the thyroid revealed enlarged parathyroid glands lying in the substance of the thyroid tissue. In 2 animals the normal structure of the parathyroid, with its diffuse arrangement of cells and occasional cords, was replaced by an adenomatous structure with hyperplasia (Figs. 4, 5).

Twenty-four of the 59 animals had been given, in addition to 2-acetylaminofluorene, measured doses of aromatic amino acids ranging between 143 and 416 mgm. in 45 days. None of those fed with amino acids

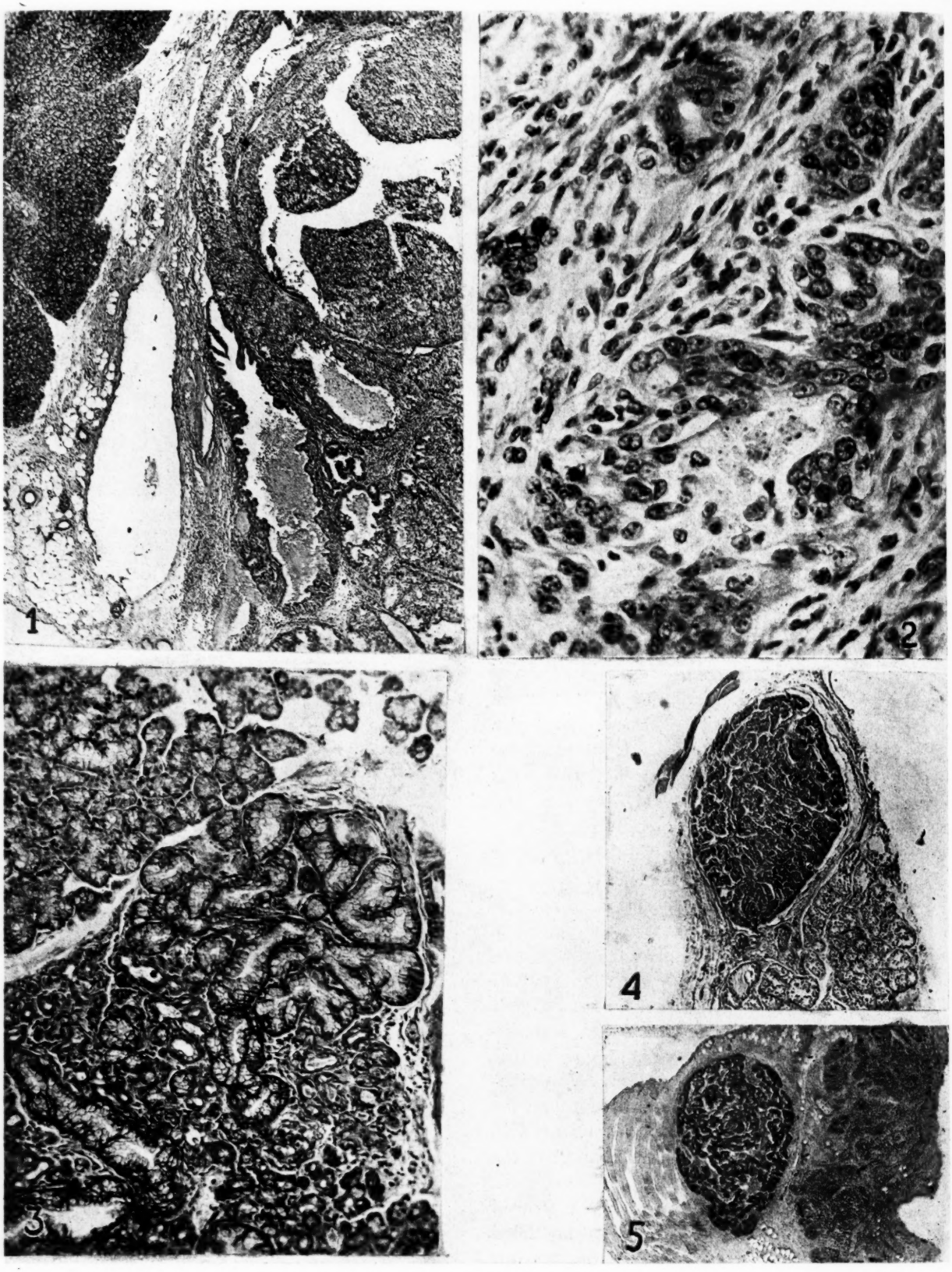

Fig. 1.—Papillary cystadenocarcinoma of submaxillary salivary gland. Normal gland, upper left. Mag. × 60.
Fig. 2.—Invasion of stroma by cancer. Mag. × 454.
Fig. 3.—Adenoma of submaxillary salivary gland. Mag. × 120.
Fig. 4.—Parathyroid adenoma. Mag. × 60.
Fig. 5.—Parathyroid adenoma. Mag. × 60.

developed any tumors after the administration of 2-acetylaminofluorene up to 650 mgm. The aromatic amino acids probably interfered with the carcinogenic activity of the acetylaminofluorene. The animals fed with aromatic amino acids and acetylaminofluorene did not develop cirrhosis of the liver as did those fed with the latter compound alone.

Thirty-six animals with transplanted mammary adenofibromas were fed acetylaminofluorene, which had no effect on their growth, and no malignant changes were noted in them.

CONCLUSION

The localization of cysts and tumors in the neck may be attributed to trauma of the pharynx and esophagus produced by the needle. The 2-acetylaminofluorene may therefore exert a local action if applied frequently to the same site. Wilson and his co-workers reported that no tumors appeared after subcutaneous injection of 2-acetylaminofluorene (3). In view of our observations it is suggested that this chemical may constitute an industrial hazard.

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Localization of Stratum of Maximum Mitotic Frequency in Epidermal Methylcholanthrene Carcinogenesis in Mice*

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The purpose of the major cancer research project of Barnard Hospital is to measure the properties of epidermis during epidermal carcinogenesis induced by methylcholanthrene, to integrate the findings and thus to construct a comprehensive account of what happens (6).

Mitotic frequency is one of these properties and has been investigated by Cooper and Reller (5) and by Reller and Cooper (12). They have observed that 18 hours after the initial application of methylcholanthrene there is a distinct increase in mitotic frequency over that exhibited by normal epidermis, that this increase grows in magnitude to 16 days, that it is maintained fairly constant to 51 days, when it decreases only to increase again about the time that cancers make their appearance in the series. Since these observations were based on counts of mitoses and of nondividing nuclei made on preparations of whole epidermis mounted with proximal, that is dermal, surface uppermost which were viewed vertically, it was not possible to identify the epidermal strata in which the mitoses occurred. The counts were therefore total for whole epidermis, not differential for its several strata.

Differential counts of mitotic frequency can be made only on stained sections of epidermis which are vertical to the surface and which cut across the strata at right angles. But the epidermis of the area chosen for treatment with carcinogen is normally so thin (2 to 3 strata) that in it the strata, easily identifiable in the early hyperplastic stages of carcinogenesis, are not represented. Consequently, it was necessary to make differential counts of mitotic frequency on much thicker epidermis of mice, which normally is stratified, in order to secure data on the basis of which to interpret differential counts of epidermis in our carcinogenic series. Cowdry and Thompson (9) selected for this purpose epidermis of the hind foot pads of mice of the same New Buffalo strain used in the major project. They produced a summation of mitoses by injecting the mice with colchicine and discovered that the maximum mitotic frequency was localized in the middle third of the suprabasal part of the epidermis.

The next step reported in this paper was, employing the same technic, to make similar differential counts of mitotic frequency in the early hyperplastic stages of carcinogenesis to supplement the total counts of Cooper and her associates. It was thought that the normal level of maximum mitotic frequency might be shifted during carcinogenesis because in a few preliminary counts Cowdry and Paletta (7) found more mitosis in the basal than in the spinous layer and also for the reason that Thuringer (18) had reported that it can be shifted in cats by the simple expedient of electrical stimulation.

MATERIALS AND METHODS

Male New Buffalo mice, 6 weeks old, were divided into two groups of 5 each. Mice of the first received on the back 3 cutaneous applications of 0.6 per cent methylcholanthrene in benzene in the uniform manner already described in numerous reports on the major project and were killed 10 days after the initial treatment; whereas those of the second received 6 applications and were killed after 20 days. Eight hours before killing, all mice were injected with 0.01 per cent aq. colchicine as prescribed by Paletta and Cowdry (10).

Uniformly hyperplastic-looking samples of skin from the treated areas were excised, fixed in Bouin's fluid and serial 6 micron paraffin sections were stained with hematoxylin and eosin. The samples of epidermis counted were carefully selected as typical of the several specimens. The thickness, of course, was in general greater in the 20 day mice than in the 10 day mice and there was a little variation, noticeable on naked eye inspection, between individual mice of the same group. Epidermis dipping into hair follicles, or unusual in respect to mechanical injury, or particularly massive leukocytic invasion, or for some other reason, was not included.

Epidermis backed by a basement membrane, which in section was approximately a straight line, proved

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most satisfactory because in it the epidermal cells were arranged with some regularity. The stratum basalis was regarded as composed of cells actually in touch with the basement membrane. Identification of such basal cells was a simple matter. It was possible to resolve the stratum spinosum into several rows of cells and to count the number of mitoses and nondividing nuclei in each. Rows of squares ruled on a disc inserted in the ocular of the microscope served as guides and prevented counting the same nuclei twice. Nuclei in the more distal stratum granulosum were counted, but were not included in Table I because none of them was in mitosis.

The procedure in making the counts was to orient the section so that the large square on the disc (itself subdivided into the small ones mentioned in the preceding paragraph) covered the stratum basalis and the

OBSERVATIONS

In the upper half of Table I are data relative to mice under treatment with carcinogen for 10 days and in the lower half to mice treated for 20 days. Passing in columns from left to right the numbers assigned to individual mice are given, then the numbers of mitoses (M) and nondividing nuclei (N) counted in the stratum basalis and in 1 to 7 rows of cells of the stratum spinosum. The figures are totaled and the ratios of the totals are listed in the last lines of the upper and lower halves of the table.

The first and most obvious fact is that the total ratios of mitoses to nondividing nuclei were greater in the stratum basalis at both 10 and 20 days than they were in any row of cells of the stratum spinosum.

It is clear also that the epidermis was in general somewhat thicker in the 20 day than in the 10 day

TABLE I: COUNTS OF MITOSIS AND NONDIVIDING NUCLEI IN EARLY EPIDERMAL CARCINOGENESIS

Mice, 10 days	Stratum basalis		Stratum spinosum													
			Row 1		Row 2		Row 3		Row 4		Row 5		Row 6		Row 7	
No.	M	N	M	N	M	N	M	N	M	N	M	N	M	N	M	N
1	52	2,573	9	1,688	1	1,311	1	829	0	533	0	111	0	14	0	0
2	34	3,241	10	1,984	2	1,261	0	498	0	140	0	30	0	3	0	0
3	79	4,202	11	2,581	0	1,418	0	264	0	9	0	0	0	0	0	0
4	49	2,691	9	1,819	3	1,178	1	437	0	72	0	7	0	0	0	0
5	65	3,752	21	2,470	6	1,562	1	505	0	51	0	22	0	0	0	0
TOTALS	279	16,459	60	10,742	12	6,730	3	2,533	0	805	0	170	0	17	0	0
Ratios	1:	58.99	1:	179.03	1:	560.83	1:	844.33								
Mice, 20 days																
1	43	4,684	16	3,359	5	2,367	3	1,284	1	427	0	86	0	13	0	7
2	32	4,017	12	2,797	3	1,759	2	916	0	338	0	93	0	24	0	1
3	66	3,679	16	2,136	0	1,013	0	229	0	20	0	2	0	0	0	0
4	35	1,544	8	1,022	4	698	1	399	0	139	0	35	0	4	0	0
5	80	3,908	38	2,940	21	2,316	15	1,678	2	928	1	341	0	80	0	21
TOTALS	256	17,832	90	12,254	33	8,153	21	4,506	3	1,852	1	557	0	121	0	29
Ratios	1:	69.66	1:	136.15	1:	247.06	1:	214.57	1:	617.83	1	557				

suprabasal layers. Since all of the included mitoses and nuclei were counted, the figures given in the table under M (mitosis) and N (nondividing nuclei in the layer of basal cells and in the rows of spinous cells (Nos. 1 to 7) give a fairly satisfactory measure of relative cellularity on the basis of one cell per mitosis or nucleus.[1] The basal cells were the smallest and therefore the most numerous in all areas of epidermis covered by the large square.

All the counts were made by B. B. G. under an oil immersion objective with a mechanical counter. It was hard work for one person but gave uniformity to the counts, which would have been lacking had several people done the counting.

specimens, for in the former only was a seventh row of cells encountered in the spinosum of 3 out of 5 mice. Moreover, the sixth and even the fifth rows were better represented at 20 than at 10 days. The mitoses were found to extend further up into the epidermis at 20 days and the ratios in the second and third rows of spinous cells were noticeably greater than at 10 days.

Though this shift was paralleled by an increase in thickness, the over-all ratio of frequency of mitosis for basal and spinous cells was decreased from (354: 37.456) 1:105.7 at 10 days to (404:45.304) 112.1 at 20 days, whereas Cooper and Reller (5) observed in their mounts of whole epidermis a ratio of 1:166.66 at 9 days and one of 1:106.9 at 23 days.

From the data presented it is a simple matter to calculate the ratios for separate mice at the various levels. The individual differences in the extension of mitosis into the several rows of the spinous layer are

[1] However the presence of epidermal cells possessed of more than one nucleus must be taken into consideration in arriving at an accurate measure of cellularity. These will soon be described by Gopal-Ayengar, A. R., "The Occurrence of Bi- and Multinucleated Cells in Epidermis." Anat. Rec. (In Press)

more clearly presented in Table II than in Table I. In 10 day mouse 3 and 20 day mouse 3 mitoses were not found distal to the first row. Variability in extension was noted in both groups of mice.

In the stratum basalis at 10 days the range was from 1:49.4 in mouse 1 to 1:94.4 in mouse 2; while at 20 days it was from 1:44.1 in mouse 4 to 1:126.1 in mouse 2. In 10 day mouse 2 the ratio of 1:63.5 in the second row of spinous cells was greater than that of 1:94.4 in the basal cells. Because of such variability we do not consider the difference in observed total ratios for basal cells between 1:58.99 at 10 days and 1:69.66 at 20 days (Table I) as necessarily significant.

DISCUSSION

Evidently this localization of maximum mitotic frequency in the stratum basalis of the epidermis 10 days after the first application of carcinogen is in interesting contrast to the localization of maximum mitotic frequency more distally in the middle third of the suprabasal epidermal layer of the hind foot pads of mice of the same strain.

But the mice, the foot pads of which were studied by Cowdry and Thompson, were younger than those used in this experiment, being only 10 days old, and it is of course possible that, had the counts been made on the foot pads of older mice, the level of maximum frequency might have been found to be more proximally situated than in the young ones. In other words, an age factor in the determination of this level may exist, but there is as yet no evidence whatever that it does exist. It is easy to be wise after the event. It would have provided a more direct comparison had Cowdry and Thompson employed 6 weeks old mice, but for us to have selected 10 day old mice, as they did, would have interfered with the directness of the comparison of the data herein described with other available data on properties of the hyperplastic epidermis 10 days after the initiation of treatment with the carcinogen. Even as it is, the evidence presented by Thuringer (6-8) that there are normally more mitoses in the spinous than in the basal strata in human beings and cats, in conformity with a similar location in young mice, suggests the probability that the usual location of maximum mitotic frequency in thick mouse epidermis is more distal than it is at 10 days in our carcinogenic series.

However this may be, proximal concentration of mitoses at 10 days is a property of the reacting epidermis contemporaneous with the presence of a new chemical equilibrium in it, details concerning which have been recently summarized (6). It is to be noted also that at 20 days the maximum mitotic frequency, still located in the stratum basalis, remained substantially the same as it was at 10 days just as the new chemical equilibrium endures without significant change for weeks. We could have made these differential mitotic counts at 30 and even at 40 days, but it would have been with increasing difficulty because the rows of cells become more irregular as hyperplasia advances and it did not seem necessary to prolong this fatiguing and monotonous kind of work. The essential task was to cover the early stages of hyperplasia during which many new properties appear, which, with remarkable uniformity, are maintained until about the time when a few cells break through the basement membrane, display malignant behavior, and form tumors.

TABLE II: INDIVIDUAL M/N RATIOS

Mice, 10 days No.	Stratum basalis	Stratum spinosum				
		Row 1	Row 2	Row 3	Row 4	Row 5
1	1:49.4	1:188.4	1:131.1	1:829.0	—	—
2	1:94.4	1:196.4	1:63.5	—	—	—
3	1:53.1	1:234.6	—	—	—	—
4	1:54.9	1:202.1	1:392.6	1:437.0	—	—
5	1:57.7	1:117.6	1:260.3	1:505.0	—	—
Mice, 20 days						
1	1:108.4	1:209.9	1:433.4	1:428.0	1:427.0	—
2	1:126.1	1:233.0	1:586.3	1:458.0	—	—
3	1:55.7	1:133.5	—	—	—	—
4	1:44.1	1:127.7	1:174.5	1:399.0	—	1:341.0
5	1:48.7	1:77.3	1:110.2	1:111.8	1:617.3	1:557.0

It is not a case of the level of maximum mitotic frequency shifting, in consequence of the applications of carcinogen, to a position in close apposition to the underlying blood vessels from a more distal location. Rather is it the expression of a tendency on the part of mitotic division to continue in the descendants of the basal cells, in which it was originally centered when the epidermis consisted only of 2 or 3 layers of cells and before it became hyperplastic. With increasing hyperplasia more cells are displayed toward the surface and some of them undergo mitosis; but, up to 20 days in our series, the level of maximum mitotic frequency is still in the basal cells. Why is this level

so proximal in location in contrast with its more distal situation in the spinous layer of normal epidermis of about the same thickness?

Perhaps the frequency of mitosis is conditioned by the action of methylcholanthrene and/or its products as mitotic stimulants or as inhibitors. Simpson and Cramer (13, 14) followed the carcinogen into the skin by fluorescence microscopy, and, by making fluorescence spectra, they noted significant changes in it. In stages of moderate hyperplasia, resulting from several paintings, the amount of fluorescent material is generally greatest in the distal strata, near the surface, and decreases as the basal layer is reached. If all of this fluorescent material is equally active carcinogenetically the basal layer may receive less of a stimulus than the spinous layer. If, further, we are dealing with an agent which in high concentration inhibits mitosis, or is less of a mitotic stimulant, the greater mitotic frequency observed in the basal layer would be understandable. This, however, is speculation. So, also, is the idea that the decomposition products of methylcholanthrene, which Simpson is detecting in the dermis, may be responsible for the greater mitotic frequency in the basal cells, which are more immediately exposed to dermal influences than the spinous ones, if, in the concentrations available to the epidermis, these products are mitotic stimulants.

It is likewise possible that the new chemical equilibrium, imposed on the epidermis in some way by methylcholanthrene and/or its products, does not influence equally the cells of the basal and spinous layers. In case the decreases in calcium, copper, cholesterol, biotin and other materials work greater hardship on the spinous cells than on the basal ones, which latter are nearer the source of supply in the vasularized dermis, living conditions compatible with mitoses may be less favorable for them than for the basal cells the higher mitotic frequency of which we are trying to explain.

More data are urgently needed on the localization of chemical alterations within the reacting epidermis. Analyses have been made chiefly of the whole epidermis of the area treated with carcinogen, indeed, usually of several specimens taken together to give sufficient amounts of tissue. The range in calcium content and in other properties between different epidermises similarly treated and between different parts of and different levels of a single epidermis, is not known.

All we have at present are certain microscopic observations of little quantitative value which suggest a greater change in the spinous than in the basal layer. These observations were that in the spinous layer the nuclear chromatin and nucleoli are more easily displaced than are those in the basal layer by equal ultracentrifugal force (8); the increase in volume of spinous cells is greater than that of basal cells (7); and that microincineration preparations sometimes show less mineral residue in the spinous cells than in the basal ones (11) suggesting that the cells of the spinous layer may be less well provided with minerals than those of the basal layer.

A major task ahead is to obtain these desired quantitative data on the individual chemical compositions of the basal and spinous layers. It will be necessary first to separate the two layers so that each may be analyzed by itself. By chemical means it is possible to split the epidermis into suprabasal and basal layers (1). This does not help much; for exposure to the solution modifies the composition of the tissue. The method holding greatest promise seems to be to stretch the skin and make thick sections of fresh frozen epidermis parallel to the surface. It might then be feasible to collect sufficient material for direct measurements of the components, calcium, cholesterol, etc., in the basal and spinous layers of the epidermis respectively. The chances are that it would be possible both to try other microchemical methods and to get contrasting data on enzyme composition of the two layers in such thick sections by Linderstrøm-Lang technics.

If evidence is secured that, during these early stages of carcinogenesis, the spinous cells are more subjected to deprivations of various sorts and are exposed to harsher living conditions than the basal cells, the fact that they show less mitotic activity than the basal cells would not be surprising. In the event of an actual struggle for existence among the spinous cells, not felt to the same degree in the basal ones, more favorably placed in respect to food supply and removal of waste, Spencer's (15) concept of "Cancer as a survival process" would receive support. There is reason to think that some cancer cells are tougher and have a greater ability to survive than normal cells from which they originate (3). Since only very few of the myriads of cells in the reacting epidermises undergo a malignant transformation, which thereafter appears to be indelibly engraved in the organization of their descendants so that by multiplication they form cancers, the chance that in our experimental series the malignant transformation is a kind of mutation, may not be lightly dismissed.

Unfortunately in the investigation of Biesele and Cowdry (2) observations of chromosome abnormalities had to be carried out on whole cells of impression preparations; because the sections, which would have given topographic information, might include only parts of the chromosome clumps. If the chromosome abnormalities are mainly in the spinous layer, as we think is the case, in view of the large size of cells and nuclei, the suggestion of a possible mutation therein would have

greater weight. Evidently before reaching a tentative conclusion, we must await not only accurate measurements of the contrasting properties of the spinous and basal layer but also more detailed microscopic observations than have yet been made on the actual location of the malignant transformation, and not be content with the tacit assumption that it is somewhere in the spinous layer.

Caution is necessary in basing conclusions on the observed differences in chemical composition of the resulting cancers and of the whole hyperplastic epidermises in which they originate. In this paper we report pronounced differences between the spinous and basal epidermal layers relative to mitotic frequency and we mention other possible differences especially in chemical composition. It is possible that the malignant change occurs in an epidermal layer, the composition of which resembles that of the cancers much more closely than does that of whole hyperplastic epidermis.

Evidently in this major project of Barnard Hospital, attempts are made to integrate observations bearing ever closer on the true sequence of events in the line of carcinogenesis; first, the properties of whole reacting epidermis; second, those of the particular epidermal layer in which the malignant transformation takes place (for the others are not in the direct line); third, those of the very few of many cells in the layer which undergo malignant change and finally, those of the components of the cells which condition their new malignant behavior. Eight years ago the keel of the project was laid; the launching is just in prospect.

SUMMARY

Of 5 male New Buffalo mice, 6 weeks old, the backs of which were treated 3 times with 0.6 per cent methylcholanthrene in benzene, 4 showed a greater mitotic frequency in the basal epidermal layer than in any of the rows of spinous cells, while 1 showed a greater frequency in the basal layer than in the first row of spinous cells and less than in the second row 10 days after the initial treatment.

Five similar mice showed maximum mitotic frequency in the basal layer 20 days after the first application of carcinogen. Though considerable variability was observed in individual ratios of mitoses to nondividing nuclei the conclusion is drawn that under the conditions of the experiment maximum mitotic frequency is predominately in the single layer of basal cells in touch with basement membranes.

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Further Studies of Specific Precipitin Antiserums for the Protein of Cancer Tissue

III. Relation of the Proteins of Different Malignant Tissues to Each Other*

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In a previous communication (1) we have demonstrated the fact that the proteins of malignant tissues are different from proteins of any of the important normal tissues of the body. In that study we used precipitin antiserums for protein of different malignant tissues and titrated these antiserums against autolysates of malignant tissues. We have discussed the difficulties attendant on the preparations of antiserums for protein of malignant tissues in our second communication (2) on this subject. With the method we have used, we have obtained only 11 per cent of potent antiserums. These antiserums were not stable for any long periods of time. We also discussed the difficulty of obtaining satisfactory malignant tissue for this work. As a result, frequently the material that we most desired to test was not available and also the reverse was frequently true that when material for testing was available we had no potent antiserum. This explains the very high percentage of blanks in our table of results (Table I). Although the results obtained are so sketchy in character that it is impossible to draw any definite conclusions, they are presented as a summary of information gained from prolonged study of precipitin reactions with autolysates of cancer tissues.

If we examine the results obtained with antiserum for the carcinoma of the breast marked 'special' (see Table I), which was a mixture of malignant tissue from a number of different specimens, we observe that when tested against 8 different autolysates, one of which was also from a mixture of malignant tissue from different specimens there was not a single positive reaction. This antiserum gave a strong reaction with the autolysates of the 'special' malignant tissue mixture. This leads us to speculate that there is probably a wide variety of proteins manufactured by the malignant cells. The results on the antiserum for carcinoma of the breast No. 126 lends support to this view, while the results on antiserum for carcinoma of the breast No. 132 shows some overlapping. The results on antiserum of metastatic carcinoma of the liver, serum No. 74[5], shows no positive reactions with the 28 autolysates tested, with the exception of the original tissue from which the antiserum was made, and that of another metastatic carcinoma of the liver, together with the autolysates of the tissue from three different carcinomas of the stomach.

The antiserum for carcinoma of the kidney No. 163[3] was tested against 27 autolysates. There were positive reactions from autolysates of 5 different carcinomas of breast. It reacted to the autolysate of the tissue from which it was prepared, reacted positively with the autolysate of one of two hypernephromas and the autolysate from one stomach carcinoma.

The antiserum for carcinoma of rectum No. 507 was tested against 19 autolysates of different malignant tissues. Fourteen positive reactions were obtained and 5 negative reactions.

On the basis of these results we are speculating that the malignant cell has lost the ability, possessed by the normal cell, of manufacturing a specific protein that is characteristic of itself, and that the particular kind of protein prepared by the malignant cell is a resultant of its environment and the forces to which it is subjected at any given time, so that one could conceive that a number of different proteins might be prepared by the same malignant cell if its environment undergoes marked changes.

The malignant tumors of the rectum are exposed to profound changes. This might explain the results obtained with the antiserum for this malignant tissue when titered against autolysates of malignant tumors from other parts of the body.

SUMMARY

Antiserums for carcinoma of the stomach, breast, kidney, rectum, and metastatic carcinoma of the liver were titered against autolysates of the breast, sarcoma of the breast, carcinomas of the cervix, carcinomas of the colon, carcinoma of the kidney, hypernephromas, metastatic carcinomas of the liver, carcinomas of the ovary, carcinomas of the stomach, carcinoma of the urinary bladder and carcinoma of the rectum. Some antiserums showed interreaction with non-homologous

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** Research Fellow in Surgery.

TABLE I: ANTISERUMS

Serum No. / Ca No.	Ca Stomach 137[13] 104	Ca Stomach 137[18] 104	Ca Stomach 147[2] 106	Ca Breast 65[4] Special	Ca Breast 126	Ca Breast 132	Metastatic Ca of Liver 100	Metastatic Ca of Liver 74[5] 0	Ca Kidney 160[3] 102	Ca Kidney 102	Ca Rectum 507 110
Ca Breast pool A				—				—	+		
Ca Breast 1				—				—	+		
Ca Breast 2				—				—	—		
Ca Breast 5				—				—	—		
Ca Breast 9				—				—	—		
Ca Breast 103					—						—
Ca Breast 104											+
Ca Breast 106					—						—
Ca Breast 110											+
Ca Breast 111	+	—		—		+	—	—	+	—	+
Ca Breast 112				—				—	+		+
Ca Breast 113		—		—		—	—	—	+	+	
Ca Breast 126					+						
Ca Breast 127					—						
Ca Breast 132					—	+					—
Ca Breast 133					—						
Ca Breast Special		—	—	+		+	—	—	—	—	
Sarcoma Breast 128											+
Ca Cervix NP		—	—			—	—	—		—	
Ca Cervix i	+			—				—	—		+
Ca Cervix ii				—				—	—		+
Ca Cervix iii				—				—	—		
Ca Cervix 100				—	—			—	—		
Ca Colon 102											—
Ca Colon 103	—										—
Ca Colon 107											+
Ca Kidney 102	—	—		—		—	—	—	+	+	
Hypernephroma 100								—	—		
Hypernephroma 101								—	+		
Metastatic Ca Liver 0		—		—				+	—	—	
Metastatic Ca Liver 100				—		—	+	+	—		
Ca Ovary i				+				—	—		
Ca Ovary 5					—						+
Ca Stomach I			—	—				+	—		+
Ca Stomach ii				—				+	+		+
Ca Stomach iii	—			—				—	—		
Ca Stomach iv				—				+	—		
Ca Stomach 100				—				—	—		
Ca Stomach 101								—			
Ca Stomach 104	+	+									
Ca Stomach 106	+	+	+	—		—		+	—	+	+
Ca Urinary Bladder 100	—	—		—		—	—	—	—	—	+
Ca Rectum i				—			—	—	—		
Ca Rectum 110											+

autolysates whereas antiserum for carcinoma of the breast special was relatively specific, as it reacted only with its own autolysate and autolysates of carcinoma of the ovary i. Some of the antiserums reacted only with their homologous autolysates.

CONCLUSIONS

1. There is probably a wide variety of proteins manufactured by malignant cells.

2. The protein prepared by malignant cells may be dependent on its environment and forces to which it is subjected.

3. The malignant cells may have lost the ability of manufacturing a specific protein, characteristic of itself.

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A Transplantable 9,10-Dimethyl-1,2-Benzanthracene Sarcoma in the Syrian Hamster*

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Comparatively little has been published on the use of the hamster in cancer research and only two reports, including some 15 instances, of spontaneous tumors are available (1, 9). The earliest experimental work appears to be Gye and Fould's (9) production of a mixed-cell sarcoma in two males by injecting 3,4-benzpyrene, in 1939. A year later Halberstaedter (11) reported the production of a similar sarcoma in 29 hamsters by repeated injections of benzpyrene and in 1944 extracts of a sarcoma produced by benzpyrene were used to study the rate of cell growth in tissue cultures (6).

No work on the use of 9,10-dimethyl-1,2-benzanthracene as a carcinogen in hamsters has been found. However, results of several investigations with mice (2, 4, 14, 15), with rabbits (3) and by other standards (12) show that this is a very potent carcinogen.

This work was undertaken to determine if the hamster is a suitable animal to use in studying the histologic structure of a tumor induced by injecting 9,10-dimethyl-1,2-benzanthracene and perpetuated by transplantation.

MATERIALS AND METHODS

Male and female Syrian hamsters (*Cricetus auratus*, or *Mesocricetus auratus*) from two geographically widely separated colonies were used in these experiments. The stock from the University of Colorado was inbred, but not consistently brother-sister mated, from two males and four females obtained from the Fairfield Rabbit Farms, Caldwell, N. J., and the other stock was from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The JAX hamsters ranged from 39 to 74 days old at time of implantation, but the Colorado animals varied from 36 to 335 days in age and included sexually immature young, adults and senescent breeding animals.

The original tumors for this work were obtained by injecting four female hamsters with 9,10-dimethyl-1,2-benzanthracene. The tumors thus derived, as well as those obtained by transplantation, were polymorphous, or mixed-cell, sarcomas.

The carcinogenic solution was made by dissolving 20 mgm. of 9,10-dimethyl-1,2-benzanthracene (Eastman Kodak Co.) in 5 cc. of olive oil and 5 cc. of benzene (Merck, thiophene freed, A. C. S.). This solution was injected into the areolar tissue under the cutaneous maximus (panniculus carnosus) muscle in the right lateral lumbar region. In the hamster, as well as in other animals having a well developed cutaneous maximus muscle, the areolar tissue in the lumbar area is an excellent site for injection because the iliolumbar artery and vein anastomose freely with 5 other important arteries and veins in this muscle and the long thoracic and internal mammary anastomose with the inferior epigastric and other arteries and veins in the body wall and thus form a high vascularity for this extensive, areolar area (here designated as the subpannicular area to avoid incorrect use of the term subcutaneous).

Each of the 4 original hamsters received 4 injections of this solution over a period of 41 days. Three injections of 0.10 cc. were made 17 and 8 days apart and one of 0.15 cc., 16 days later. Three of these animals supplied the original tumors for all the transplantations; while the fourth was allowed to develop an extensive lung metastasis.

The transplantations were effected either by grafting, in which a wedge-shaped piece of solid tumor or a mass of soft material from a necrotic growth was inserted subpannicularly with concave forceps or by injection, in which fluid and solid material from an incision in the tumor were drawn through an 18-gauge needle into warm salt solution and injected into one or both lateral lumbar regions. Usually, transplantations, were made to one side of the mid-dorsal line, but in some cases a single graft was inserted subpannicularly into the mid-lumbar region; while in others transplants were made by grafting into one side and injecting into the other (Fig. 2).

Transplantable metastases to lymph nodes, lungs and kidneys were obtained by subtotally removing the sarcoma once or twice and allowing the new growth to develop until the animal became moribund before it was killed. At each removal a small piece of the neoplasm was left *in situ* to insure early recurrence.

The 6 principal lines of this sarcoma, all derived from one benzanthracene-induced tumor (Fig. 1,

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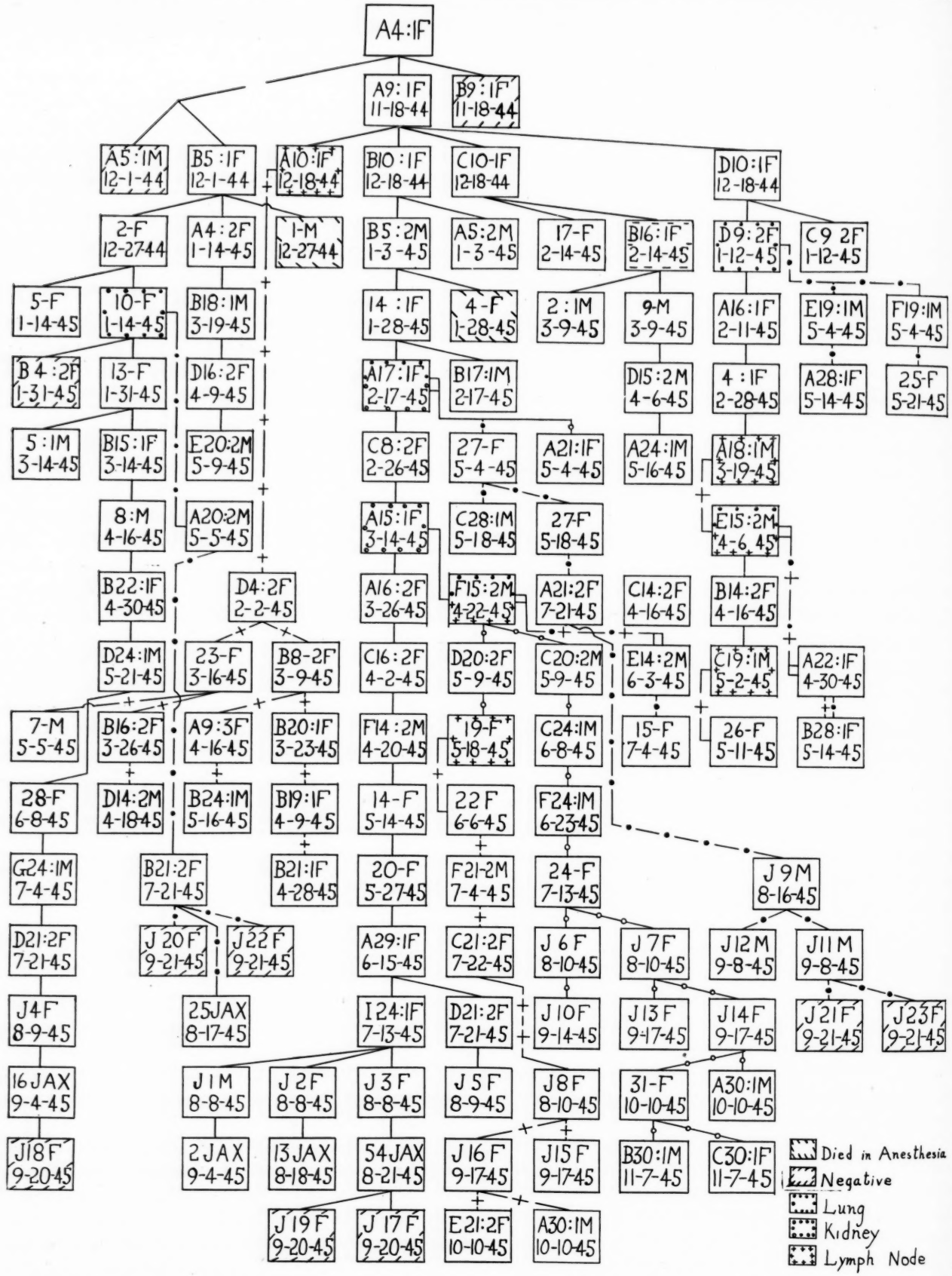


FIG. 1.—Generations of transplants from a benzanthracene-induced tumor to show main lines and lines derived from metastases to lymph nodes, lungs and kidneys. Transplants of organs made purely as tests for metastasis are not included. Each of the boxes for the 234 transplants includes the symbol of a single host and the date it was implanted, except that the numbers preceding the letters JAX denote the total number of individuals receiving transplants from a single donor; thus, the symbols 2-, 13-, and 54 JAX represent transplants to 110 JAX hamsters from 5 different donors. The letter J denotes JAX stock, all the others represent Colorado stock. Failure of the 7 JAX hosts to take was the result of an accident.

A4:1F), that were transplanted to both Colorado and JAX hamsters included the following lines: (a) Main line (Fig. 1, A4:1F through J3F), 17th passage; (b) second donation of A4:1F (Fig. 1, B5:1F to J18F), 13th passage; (c) Metastatic kidney (Fig. 1, A15:1F through C30:1F), 12th passage; (d) Metastatic lymph node (Fig. 1, 19-F through E21:2F), 12th passage; (e) First metastatic lung (Fig. 1, A17:1F to J21F), seventh passage; and (f) Second metastatic lung (Fig. 1, 10-F through 25 JAX hosts), 4th passage.

All transplantations, including injections, were done under nembutal anesthesia in which a 2 per cent solution was injected subpannicularly in dosages carefully estimated at 0.04 ml. to 10 gm. of body weight for males and females weighing 80 to 115 gm.

Benzanthracene-Induced Tumors

No symptoms of pain were apparent in any of the 4 animals following the first three injections of the carcinogenic solutions, but 41 days after the first injection three of the hamsters had the skin adherent at the site of the injections. The fourth injection in each animal was, therefore, made immediately crainiad to the adhesion. Eleven days after the last injection (52 days from the first) two of the hamsters had developed lesions at the site of the last injection. Another developed a similar lesion 2 or 3 days later, but there was no lesion in the fourth animal and the tumor appeared on the side opposite the side of injection. Another animal not only developed a lesion and a tumor at the site of the injection, but a second one about 15 mm. in diameter was found on the abdomen 25 days after the first appeared.

Tumors were first noted in each animal 77, 81, 92, and 95 days following the first injection of the carcinogen. These tumors grew rapidly and attained a diameter of about 5 cm. before showing signs of necrosis.

All four of the original 9,10-dimethyl-1,2-benzanthracene-produced tumors became enormous in size without affecting the general physical condition of the hamster. One animal evidenced no symptoms of pain and ate heartily 127 days after the first injection, but became definitely moribund and was killed 6 days later. At this time the original tumor extended, in the form of an exaggerated oval, from low on the left ischium across the back to the right scapula; while a second tumor adhered to and protruded fully 12 mm. from the abdominal wall.

Two hamsters that had the primary tumors removed 89 and 98 days following the first injection of benzanthracene and were killed 10 and 13 days after the operation had no macroscopic metastases. Although growth had been very rapid, the tumors apparently had not had time to metastasize.

The tumor of one of these hamsters was transplanted at the time of subtotal extirpation into two hosts one of which died in anesthesia and the other, a sterile female, was negative after 21 days when she was again grafted with the regenerated tumor of the same donor with two other hosts which developed normal takes. The tumor in this sterile female was first noticed 10 days after transplantation. It was very small, attached to the body wall, and continued to develop much more slowly than those in the other 2 hosts. It was transplanted at 77 days and developed normally during the next 5 passages, with a minimum of 16 and a maximum of 26 days between transplantations. The tumor in a male, which was grafted at the same time as the sterile female, was passed through 5 transplantations with a minimum of 17 and a maximum of 44 days between passages, thus indicating that environmental conditions, rather than a different strain, was the cause of the delay in growth of the tumor in the sterile female.

The other two hamsters were allowed to live unmolested until one was found dying of its tumor and the other was moribund, when they were killed. The first hamster, which had lived 125 days from the first injection, showed gross macroscopic metastases to the lungs. The other animal, which lived 135 days from the first injection, showed macroscopic metastases to the exterior of the abdominal musculature, retroperitoneum, broad ligament, left superficial inguinal lymph node and lungs. The microscopic structure of the metastases is essentially the same as that of the primary tumors.

Transplanted Tumors

Injections of the viscous white or red, and of aqueous liquid material from soft tumors were approximately as capable of transferring the tumors as grafts of solid tissue and had about the same period of latency. Of 23 hamsters doubly implanted with injections of liquid necrotic tumors all were takes on one or both sides. Complete records of the interval between implantation and definite take, although available for only 7 of these animals, ranged from 7 to 8 days in both sides, except that in one animal the right side was negative.

Latency.—The time required for development of the tumor was found to differ according to the method of transplantation used. Transplants made by injecting blood from solid tumors commonly were about 10 days later in developing than those made by grafting tissue (Table I). However, if the walls of the incision in a solid tumor were gently scraped with a scalpel and particles of the tumor withdrawn and injected with the blood a noticeably higher percentage of takes was obtained and the resulting tumor developed about as

rapidly as if a piece of solid tissue had been grafted. This condition indicates an insufficient dosage (5), rather than an infection or delay of the tumor cells in reaching healthy tissues, as Haddow (10) suggests.

The time interval between implantation and take, as indicated by palpable, solid enlargement of the implant, is more closely related to the volume than to the the tumor were included in the blood injection the latent period of takes and the number of failures were reduced to about the level of those obtained by grafting. Unusually large quantities of tumor failed to shorten the latent period for takes over those obtained by optimal quantities of tissue, but did affect the host adversely, especially when necrotic material was im-

TABLE I: EFFECT OF VOLUME OF TRANSPLANTS ON TAKES

The grafts of solid tumor always contained an optimal or greater volume of carcinogenic material while injections of blood from incised solid tumors frequently contained an insufficient volume

Group and number of hamsters	Implantation to termination of experiment, days	Grafted tumor, maximum diameters, in mm.	Injected tumor, maximum diameters, in mm.
A, 12	9-19 (av. 15.0)	25 × 27—35 × 55 (av. 28.6 × 36.8)	3 × 3—25 × 35 (av. 12.0 × 16.0)
B, 10	22-47 (av. 28.9)	23 × 35—40 × 43 (av. 30.9 × 40.2)	5 × 5—35 × 43 (av. 15.9 × 20.1)
C, 12	13-77 (av. 29.7)	20 × 15—43 × 45 (av. 29.0 × 36.1)	0 0

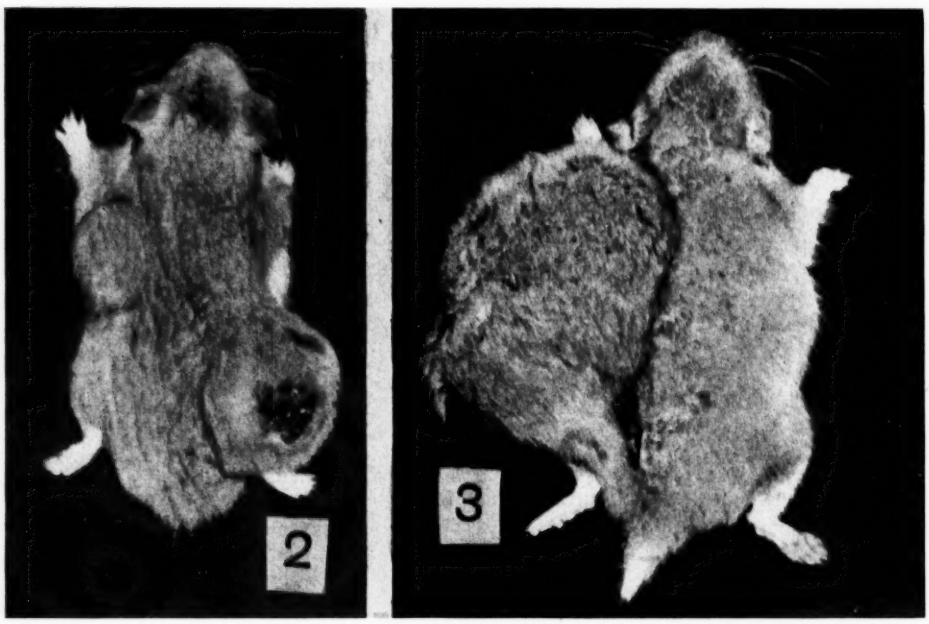


FIG. 2.—A male hamster that died 25 days after being injected with tumor-blood (left side) and grafted (right side). The injection site is unusually far forward.

FIG. 3.—An anesthetized female hamster 56 days after being injected with tumor-blood, showing a palpably solid sarcoma that had metastasized to the lungs and formed deep cell-aggregates and thrombi. The tumor weighed 16.5 gm. more than the host, and sections showed many necrotic areas.

physiological condition of the implanted tumor or to the method of implanting it. Thus, if the volume of the transplant were optimal in size, takes were obtained about as readily with injections or grafts of necrotic as with grafts of solid, actively proliferating tissue. Injections of blood taken from incisions in solid tumors invariably gave delayed takes with an appreciable number of failures (Table I), but after the period of latency these tumors often grew more rapidly than those derived from grafts. If a number of particles of planted. However, only 10 of the total of 311 implanted animals died of tumors.

Ulceration.—Tumors derived by grafting the transplant frequently ruptured at the site of the graft before any considerable amount of necrotic fluid had accumulated; while tumors derived by injecting blood or necrotic fluid often did not rupture throughout the entire experiment (Fig. 3). When cut open, each tumor 25 mm. or more in maximum diameter was found to contain a quantity of necrotic fluid which

was surrounded by a thin layer of solid, growing tissue in some instances.

Grafted tumors sometimes covered more surface of the host's body than injected tumors but seldom attained as great thickness because of the readiness with which they ruptured at the site of the graft. When a tumor ruptured, the host commonly removed the necrotic material and overlying skin thus leaving a large crater in the foul-smelling tumor.

Tumor weight.—The respective weights of the tumors and of the carcass after the tumors had been removed in 7 hamsters that had been implanted by injections were 23.3, 36.5; 65.5, 85.5; 40.5, 62.5; 33.5, 36.0; 61.8, 62.0; 68.5, 52.0; and 75.0, 46.5 gm. Six of these animals had definite metastases.

Biopsy and recurrence.—It was noted that in those instances in which total extirpation of the two transplanted tumors was attempted, the tumor from which transplants had been taken or the one that had been ruptured during removal commonly recurred; while the one that had been removed intact did not recur. Although this observation is confirmed by records on only 9 hamsters, it indicates that in this particular sarcoma biopsy will usually assure recurrence of the tumor after it has been removed. Recurrence does not appear to be a characteristic of this tumor as has been reported for the Flexner-Jobling carcinoma (7).

The escape of necrotic fluid during removal of a soft tumor was usually followed by a rapidly spreading, confluent growth over most, if not the entire, surgical area and often covered most of the back and sides of the animal. Flushing the surgical area with salt solution failed to reduce or localize this condition appreciably.

Specificity

When this tumor was transplanted from hamsters into mice of different strains, sex and ages the implant persisted and simulated a take for several days, but in each instance it was resorbed within 6 to 57 days. The 35 hosts comprised 14 heterozygous white, 4 young adult dba, 4 nursing, 2 old adult and 11 young adult C3H high-tumor, homozygous mice.

This sarcoma grew as readily when transplanted from Colorado hamsters to JAX hamsters and vice versa as it did when transplanted within either colony, that is, when meeting Loeb's (16) requirements for a strictly syngenesioplastic transplantation. Thus, each of the 34 JAX hamsters (30 of which were doubly injected and 4 singly grafted) that received transplants directly from the Colorado donors of the 6 lines of tumors developed the transplants.

In one experiment with 100 JAX hamsters (44 males and 56 females, comprising 12 entire litters and 2 individuals from another) 25 received transplants of a metastatic lung line tumor directly from a Colorado donor (Fig. 1, B21:2F) and 75 received transplants of the main line tumor from 2 JAX hamsters (Fig. 1, J2F and J3F) which had received the tumor directly from a Colorado donor (Fig. 1, I24:1F).

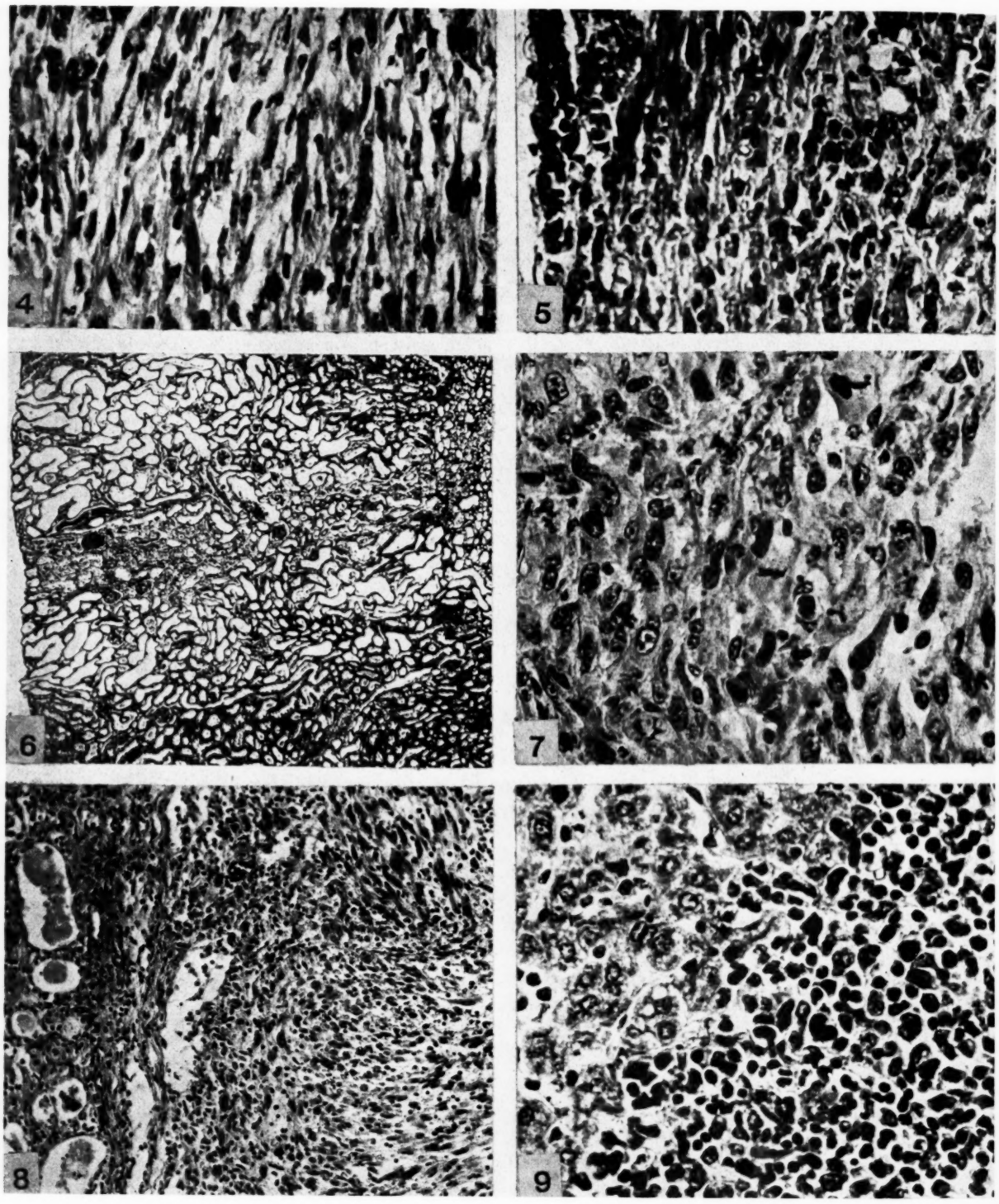
In this experiment 65 of the hosts received double injections containing fragments of the tumor and the remaining 35 received single, central grafts. The experiment was terminated 23 to 26 days after the transplantations were completed with the following results:

99 of the 100 hosts had definite takes
39 males and 49 females (34 singly grafted and 54 doubly injected) developed definite tumors within 9 days after implantation
35 singly grafted hosts had definite takes in each animal
1 of the doubly injected hosts was negative 37 days after implantation, when it was killed and examined
2 female hosts had tumors removed 6 days after being inoculated (one had been doubly injected), the other singly grafted)
5 had died of their tumors
7 had solid tumors less than 25 mm. in maximum diameter (5 doubly injected and 2 singly grafted hosts)
8 doubly injected hosts had one side negative
61 of the hosts had necrotic tumors more than 25 mm. in maximum diameter
26 of the 61 hosts had one or more enlarged deep axillary or superficial inguinal lymph nodes
4 of the 61 hosts had metastases to the lungs
31 of the 61 hosts (15 males and 16 females) had no macroscopic metastases to deep axillary or superficial inguinal lymph nodes, lungs, kidneys, liver or spleen, although the condition of the tumors was favorable for metastasis to one or more of these organs
28 of the 99 hosts had solid tumors more than 25 to 55 mm. in maximum diameter, but no metastasis, as was expected.

There was no sexual difference in response to implantation in either colony of hamsters. Of 10 litter mates in the group of 100 JAX hamsters, all implanted at the same time and with the same tumor, 2 males and 1 female had well formed tumors 9 days later; while 3 other males and 4 females were negative, as judged by palpation, on the ninth and again on the 13th day after implantation, but all 7 had definite tumors when killed 26 days after implantation. In the remaining 90 hamsters of this group 39 males and 49 females had definite tumors 9 days after implantation; while 1 male was negative 13 days after implantation, but had a well defined tumor when killed 26 days after inoculation and one female did not take at all.

Metastases

As a test for metastasis some of the suspected organs were transplanted. Thus, pieces of 4 different livers with areas having macroscopic appearance of early metastasis were transplanted, but all 4 failed to take. A single graft from each of 3 kidney metastases was a definite take and one (Fig. 1, F15:2M) is now in the



FIGS. 4-9

fifteenth passage. Of 10 enlarged deep axillary lymph nodes from donors having subpannicular implants 8 produced definite sarcomas when transplanted and one of these (Fig. 1, 22-F) was transplanted through 13 passages and is still going. A single graft from each of 9 lung metastases was a definite take and one of these (Fig. 1, 27-F) was transplanted through 5 passages before being lost by accident. Another line of metastasis to the lungs (obtained from A30:1M, Fig. 1) is now in the sixth passage.

It was early apparent that in most instances necrosis of the tumor would be followed by metastasis unless the toxicity of the necrotic material was sufficient to kill the host before the metastatic material had time to become organized into a demonstrable neoplasm. In this work the tumor was considered as being necrotic if it was open or if it was found to be soft or to have a soft spot when palpated. Thus, when a palpably solid sarcoma having a maximum diameter of 25 mm. or more was cut open macroscopic necrotic spots were usually found within it; while much smaller solid tumors commonly showed miliary ulceration when sectioned. Other conditions, such as those inhibiting, or at least not favoring, entrance of metastasis-producing material into the circulatory system, are probably responsible for the prolonged interval between implan-

TABLE II: RELATION OF CONDITION AND DURATION OF TUMOR TO METASTASIS IN HAMSTERS NOT SUBJECTED TO SUBTOTAL REMOVAL OF SARCOMAS

Animals in group E were implanted with involved organs, 2 with lymph node, 2 with lung, and 1 with kidney; group Ee, 1 with lymph node and 1 with lung

Group and number of hamsters	Implantation to death, days	No. hamsters 1 or all tumors necrotic	No. hamsters all tumors solid	No. hams. node enlargements	Lung metastasis	Liver metastasis	Kidney metastasis
A, 10	15-21 (av. 18.5)	10	0	7	4	0	0
Aa, 15	14-21 (av. 18.0)	12	3	0	0	0	0
B, 37	23-26 (av. 24.3)	32	5	33	7	3	0
Bb, 54	23-26 (av. 24.1)	27	27	0	0	0	0
C, 7	27-32 (av. 28.6)	7	0	4	2	1	0
Cc, 7	27-35 (av. 30.7)	7	0	0	0	0	0
D, 9	36-81 (av. 47.1)	8	1	5	4	2	1
Dd, 8	37-74 (av. 52.1)	7	1	0	0	0	0
E, 5	18-77 (av. 36.2)	5	0	4	2	0	0
Ee, 2	17-34 (av. 25.5)	2	0	0	0	0	0

TABLE III: EFFECT OF A SINGLE SUBTOTAL REMOVAL ON THE RELATION OF DURATION AND CONDITION OF THE TUMOR TO METASTASIS

Animals in group H were implanted with involved organs, 3 with lymph nodes, 1 with kidney, and 1 with lung

Group and number of hamsters	Implantation to first subtotal removal	No. hams. 1 or all tumors necrotic	No. hams. all tumors solid	Implantation to death, days	No. hams. 1 or all tumors necrotic	No. hams. all tumors solid	Node enlargement, 1 to 4	Lung metastasis	Liver metastasis	Kidney metastasis
F, 9	7-16 (av. 11.8)	7	2	19-42 (av. 33.7)	7	2	6	6	2	1
Ff, 10	6-17 (av. 12.6)	5	5	18-50 (av. 32.9)	9	1	0	0	0	0
G, 17	17-40 (av. 26.5)	14	3	30-88 (av. 58.7)	16	1	10	10	9	3
Gg, 4	25-49 (av. 31.7)	3	1	37-71 (av. 53.0)	3	1	0	0	0	0
H, 5	10-18 (av. 13.8)	5	0	26-43 (av. 30.8)	3	2	3	2	1	0

DESCRIPTION OF FIGURES 4 TO 9

The external side of the growth is toward the left in each figure

FIG. 4.—A benzanthracene-induced sarcoma, second donation of hamster A4:1F (Fig. 1). Mag. × 400.

FIG. 5.—Secondary sarcoma from the mediastinal wall, fifth passage, hamster A17:1F (Fig. 1). Mag. × 400.

FIG. 6.—Left kidney (from which Fig. 8 was developed), hamster A17:1F, 39 days from the second subtotal removal of the tumor. The neoplasm extended from the periphery of the organ through an area of atrophied glomeruli and tubules into the medulla. A deeply stained, nearly normal glomerulus has survived within the dense part of the growth. Mag. × 35.

FIG. 7.—Sarcoma in A21:1F produced by a graft from the right kidney of A17:1F. Mag. × 400.

FIG. 8.—Sarcoma produced by a graft from the left kidney of A17:1F (Fig. 6) into A21:1F. Note persistence of renal tubules in the graft. Mag. × 47.

FIG. 9.—Area infiltrated by small lymphocytes and cancer cells in a spotted liver 18 days after the host (fifth passage in another benzanthracene-produced tumor line) was grafted. No other metastasis was found. Mag. × 400.

tation and death in those hosts in which the neoplasm was definitely necrotic, but metastasis could not be found.

The material in Tables II and III indicates that a relationship exists between the duration and extent of necrosis and the duration of the tumor to the formation of metastases, but the data do not support this tenet sufficiently to enable one to draw conclusions. However, Haddow (10) found a significant correlation between the size and duration of the primary mammary tumor and occurrence of metastases in mice. In groups A and Aa the interval between implantation and death is too short (14 to 21 days) to be significant. In groups B and Bb, although the interval is short (23 to 26 days), 32 of the 37 hosts having enlarged lymph nodes or metastases (86.5 per cent) had definitely necrotic growths; while in the 54 non-metastatic animals of group Bb, that had the same interval, only 27 (50 per cent) had necrotic tumors when killed.

Although the number of instances is not significant, the results shown by groups E, Ee and H indicate that grafts of metastatic organs are more potent as producers of necrotic tumors than are the implants from transplanted tumors and at least as potent as producers of metastases.

Extending the interval between implantation and death by performing two subtotal removals of tumor not only produced metastasis in each of the 7 animals so treated but also resulted in multiple metastases in 5 of them. Two of these 5 had metastases to the lungs and both kidneys and the other 3 had metastases to the lungs, and enlarged lymph nodes. One pair of kidneys was not sectioned, but histologic examination showed definite metastasis to all the other organs. The intervals for these 7 hamsters were as follows: Implantation to the first subtotal removal of the sarcomas, 9 to 44 (average 24.0) days; first to second subtotal removal, 7 to 45 (average 17.6) days and implantation to death, 46 to 134 (average 80.1) days. In every instance the tumors were definitely necrotic at each of the 2 subtotal removals and at death of the animal. Thus, each hamster was exposed 3 times to actively necrotic conditions superimposed upon the potential duration of the neoplasm.

Histology of Metastases

Histological variation from the benzanthracene-induced type of sarcoma through the various passages and various metastases was no greater than could be demonstrated in a single section of this sarcoma, with the possible exception of the metastases to the liver and kidneys.

Lymph nodes.—All the enlarged lymph nodes that were sectioned varied from a condition in which the distended subcapsular and medullary sinuses contained an unusual number of monocytes and small lymphocytes to more advanced conditions in which tumor cells had infiltrated the node (verified by Dr. Cloudman and Miss Fekete) as described for mouse sarcoma 37 (17, 19) and spontaneous mammary carcinoma (17). Later, definitely sarcomatous changes including stages of obliteration of all characteristics, as described in nodes of mice implanted with sarcoma 37 and Mal. sarcoma (17) and hamsters implanted with strain H. M. of a benzpyrene-induced sarcoma (11), appeared. In very advanced stages the metastasis was indistinguishable from the primary sarcoma, and all characteristics of the lymph node had been eliminated. During the stages of altered nodal tissue and loss of the capsule, characteristic streams of large spindle cells appeared.

The number of instances of metastases to lymph nodes in which the implant had failed to take on one side or had been made on one side only, rather than in the middle or on both sides of the lumbar region, is too small to be significant. However, in an animal that died 47 days after implantation, the right side (tumor-blood injected) was negative but the right superficial inguinal lymph node was so much enlarged that sections measured 6 mm. in diameter. This node had been converted into a neoplasm without a trace of lymphoid tissue or of the capsule remaining; while on the left (the tumor-bearing side) the superficial inguinal and deep axillary nodes were enlarged only about twice, but each contained tumor cells. None of these lymph nodes was adjacent to the sarcoma.

In an effort to gain information on the route of dissemination, tumor substance was injected into the anterior jejunum with the result that a female died 14 days later of a sarcoma that completely encircled and strangled the intestine at the site of injection, and one of the three lymph nodes that comprised the pancreas Aselli contained tumor cells. A male became moribund and when killed, 27 days after implantation, two sarcomas, each about 35 mm. in diameter, were found. One of these was at the site of injection and encircled the jejunum; while the other was on the ileum 10 mm. from the cecum. One lymph node of the pancreas Aselli had been almost entirely converted into a sarcoma (verified by Dr. Mugrage) and another contained numerous large and small tumor cells.

Since lymphatic drainage of the jejunum is through the pancreas Aselli; while the venous drainage is via the hepatic portal system, it appears that these two are clear-cut instances in which a sarcoma was disseminated via the lymphatics and produced a true metastasis in a regional lymph node.

Lungs.—In every instance of metastasis to the lungs histological examination verified the macroscopic determination. In early stages the metastatic neoplasms

were commonly of four types: (a) pointed, (b) fungiform (both protruding through the pleura of the lung, the latter spreading over but not adhering to the pleura), (c) thrombi, and (d) deep cell-aggregates. All four types were readily detected and were often concurrent in the same lobe. In advanced stages all four types had been obliterated by fusion.

Liver.—Only early stages of dissemination to the liver were obtained. In these the surface of the organ appeared to be speckled or spotted with lighter color, but when pieces of the mottled livers were sectioned, they showed deep and superficial areas densely infiltrated by small lymphocytes, with a few large and small round tumor cells, as may be expected from interpretation of other works on the relation of lymphocytes to the development of neoplasms (13). In some livers there were instances of fusion of the lymphocyte-aggregates to form irregular patches (Fig. 9).

Kidneys.—Although takes were obtained from each of the 3 kidneys from which transplants were made, histologic structure of the metastasis was not clearly defined. In each instance the metastasis arose in the caudal end of the organ and extended craniad along its ventrolateral margin.

In animal A17:1F (Fig. 1), which was killed 46 days after the second subtotal removal, both kidneys showed macroscopic lesions and a piece of the right and of the left organ was transplanted to the corresponding side of the host (A21:1F, Fig. 1) where each graft produced a sarcoma. When the host was killed, 26 days after implantation, the left, a solid tumor (Fig. 8), was 15 mm. and the right, a necrotic, open tumor (Fig. 7), was 22 mm. in diameter.

The renal tubules in both the living kidney (Fig. 6) and in the left transplant (Fig. 8) had resisted destruction and contained viable nuclei, but the right graft, including the adjacent, necrotic tissue, had been removed by the host.

Subserous structures.—Metastases to the body wall, mesenteric stroma and other structures covered by serous membranes were fairly common and were invariably mixed-cell sarcomas (Fig. 5).

SUMMARY

1. The hamster is capable of 100 per cent susceptibility to the carcinogen used, as indicated by 4 animals, and to implants of the resulting tumors, as indicated by transplants to 311 individuals of various ages and both sexes.

2. The percentage of takes was as high in reciprocal transplants between JAX and Colorado hamsters as within either colony, but transplants from hamsters to 35 mice failed to take.

3. Transplants of growing and of necrotic tumors and of injections of liquid necrotic material usually developed definite takes in 4 to 10 days, but this latent period was commonly increased 8 to 10 days and the number of takes reduced about 50 per cent by injecting blood from incised solid tumors. Scraping the walls of the incision so that particles were aspirated and injected with the blood brought the latent period and percentage of takes to nearly the same level as that of the grafts.

4. Variation in latency of takes, rate and volume of growth, toxicity and metastatic potency in subpannicular transplants could not be maintained in a series of passages.

5. Tumors induced by benzanthracene, those that had been transplanted several generations and those resulting from grafts of metastases to lymph nodes, lungs and kidneys were about equally potent in producing metastases.

6. Metastases occurred in hamsters which had not been operated upon, but subtotal removal of the sarcoma 1 or 2 times greatly favored metastasis by prolonging the life of the host and thereby increasing the duration of the effects of necrosis of both the primary and recurrent tumors.

7. In 9 animals having similarly injected implants on either side complete removal of the sarcoma from which transplants were taken immediately before removal of the tumor was followed by recurrence, whereas the sarcoma of the opposite side, which had been removed intact, did not recur.

8. No permanent histologic changes were observed in 12 lines of transplants, including 8 of metastases to lymph nodes, kidneys and lungs, which were carried through 4 to 16 passages.

9. Histologic differences found in some of the newly formed metastases in the lungs, kidneys and subserosa appeared to be transient, for all well-developed primary tumors and advanced metastases had reverted to the mixed-cell type of the benzanthracene-induced sarcoma.

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Abstracts

Reports of Research

Carcinogenic Substances from Pituitary Glands of Cattle. WACHTEL, H. K. [Cancer Research Labs., Fordham Univ., New York, N. Y.] *Science,* **103**:556-557. 1946.

Anterior and posterior lobes of cattle pituitaries were extracted with acetone, ethyl ether, and alcohol. The brownish oil obtained in this way was suspended in sweet almond oil and given in amounts ranging from 10 mgm. to 30 mgm. to each of 67 white mice by single subcutaneous injection. A total of 18 mice developed malignant tumors some months after injection. Four of these were at the site of injection and the remaining 12 were found in the mammary gland, lung, and liver respectively. The majority of the tumors were carcinomas (the author states that two tumors developing at the injection sites were sarcomas and refers to all of the others as either carcinomas or cancers). Anterior lobe extracts elicited 9 tumors after an average latent period of 9.3 months. Posterior lobe extracts elicited 9 tumors after an average latent period of 14 months. Aside from this difference in latent periods the carcinogenic activity of the two types of extracts appeared to be similar.—R. B.

Réduction de l'activité d'un hydrocarbure cancérigène par un autre hydrocarbure associé. [Reduction in the Activity of Carcinogenic Hydrocarbon by Association with Another Hydrocarbon.] LACASSAGNE, A., BUU-HOI, DAUDEL, R., and RUDALL, G. [Inst. of Radium and Chem. Lab. of the Polytechnic Sch., Paris, France] *Compt. rend. Soc. de biol.,* **138**:282-284. 1944.

Two different preparations were used, the 1,2,5,6-dibenzanthracene (0.3%) and the same mixed with 1,2,5,6-dibenzacridine (0.3%). Each of these 2 preparations was injected subcutaneously into 2 groups of 10 female mice of the strain RIII. The results were similar to those obtained in previous experiments with other substances, *i.e.,* the mixture of the 2 substances provoked a loss of weight, which was less marked, death occurring later and the loss of hair not being as extensive as with the dibenzanthracene alone. A tentative explanation is given concerning the mechanism of the action of carcinogenic substances.—R. J.

Action du méthylcholantrène sur la peau préalablement soumise à une forte irradiation ultraviolette chez des souris nouveau-nées. [Action of Methylcholanthrene on the Skin of New-Born Mice Heavily Irradiated with Ultraviolet Rays.] LACASSAGNE, A., and LATARJET, R. [Pasteur Inst., Paris, France] *Compt. rend. Soc. de biol.,* **138**:351-353. 1944.

After a strong irradiation and subsequent necrosis of the skin of newborn mice certain facts were established. (1) There is an increase in the mitosis among the non-irradiated cells surrounding the necrotic zone, and (2) the formation of new hair follicles in the irradiated zone at the time of epidermic regeneration. When methylcholanthrene is applied to such areas after regeneration of the epidermis, tumors appear in the irradiated zone as well as in the surrounding area. When paintings are started before the beginning of the healing process, there is no interference with the process of healing, and there is no formation of new hair follicles, and no cancer appears in the center of the irradiated zone. Cancer may develop in the peripheral zone at an earlier time than in the surrounding healthy skin also subjected to the action of the methylcholanthrene.—R. J.

Some Effects of Aromatic Hydrocarbons on Sulphur Metabolism and Tumor Induction in Mice. CRABTREE, H. G. [Imp. Cancer Res. Fund, London, England] *Cancer Research,* **6**:553-559. 1946.

The partition of sulphur in the urine of mice receiving skin applications of benzene, naphthalene, anthracene, phenanthrene, 3,4-benzpyrene or 1,2,5,6-dibenzanthracene was measured. The noncarcinogenic hydrocarbons produced variations in sulphur distribution anticipated from previous work in which animals received these substances in their food. By contrast, the two carcinogens produced no detectable changes in sulphur distribution, indicating that mercapturate formation plays no part in their detoxication. Parallel results were obtained by measuring the effect of the same hydrocarbons on the glutathione content of the skin. The non-carcinogenic hydrocarbons are partly detoxified by a mechanism involving sulphur in the skin itself. The carcinogenic hydrocarbons produce no corresponding effect.

Naphthalene, anthracene, and phenanthrene inhibit the rate of induction of skin tumors in mice by carcinogenic hydrocarbons.—Author's abstract.

Thyroid Adenomas in Rats Receiving Selenium. SEIFTER, J., EHRICH, W. E., HUDYMA, G., and MUELLER, G. [Wyeth Inst. of App. Biochem., Philadelphia, Pa.] *Science,* **103**:762. 1946.

White rats which were given 0.05 to 0.1% *bis*-4-acetamino-phenyl-selenium dihydroxide in the diet showed increased size, hyperplasia, and loss of colloid in the thyroid glands after 10 days. After 105 days on a diet containing 0.05% of this selenium compound animals were found to have multiple adenomas of the thyroid glands and adenomatous hyperplasia of the liver.—R. B.

Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington, D. C.

Hypophysectomy in Mice with Special Reference to Mammary Cancer. A Report on the Outcome of 351 Operations. KORTEWEG, R., and THOMAS, F. [Netherlands Inst. for Cancer Research, Amsterdam, Holland] *Cancer Research,* **6**:385-395. 1946.

During the years of 1937 to 1940 investigations were undertaken to ascertain (a) the fate of mice with either high or low predisposition to mammary cancer following hypophysectomy, and (b) the fate of mice with a high cancer incidence bearing transplanted hypophyses from animals having a low rate of spontaneous carcinoma, and vice versa.

Of 350 female mice hypophysectomized, 238 survived for more than 20 days; 226 (67%) of these were considered successfully operated upon, hypophysectomy in the remaining 12 being incomplete. Microscopic examination of the ovaries of 32 mice revealed no definite corpus luteum or luteinization of the interstitial ovarian parenchyma. Hypophysectomized mice usually showed a decrease in body weight 4 to 10 months after operation, whereas in those that received hypophyseal implants it increased slightly.

In the high mammary cancer strains hypophysectomy resulted in a low incidence of carcinoma. On the other hand hypophysectomized individuals from either high or low cancer lines were unaltered at far as tumor incidence was concerned by the subcutaneous implantation of hypophyses of other mice.—E. B. B.

Influence of Estrone, Progesterone, and Stilbesterol on Growth of Lupinus Seedlings. MACHT, D. I. [Sinai Hosp. Labs., Baltimore, Md.] *M. Rec.,* **159**:164-166. 1946.

The effects of crystalline estrone, progesterone and diethylstilbesterol on the root growth of *Lupinus albus* seedlings are reported. While estrone stimulates, progesterone inhibits root growth; diethylstilbesterol was slightly inhibitory. Estrone-progesterone and estrone-stilbesterol mixtures synergistically stimulated growth of the root tip, while stilbesterol-progesterone combinations were neutralized in their toxicity for root growth.—V. J. L.

The Age Factor in Adaptability of a Sarcoma Virus to Other Animal Species. DURAN-REYNALS, F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Science,* **103**: 748-749. 1946.

Previous work by the author has shown that chicken sarcoma viruses can be adapted to ducks only when the latter are very young. In the present paper it is shown that the age of the chicken bearing the tumor is also a factor influencing the adaptability of the virus.

Tumors grown in chicks 2 to 4 weeks of age can be transferred readily to ducklings and carried in them for a maximum of 6 transplant generations after which further transplants fail to grow. The virus is not altered during this sojourn in a foreign host for when inoculated back into chickens it produces the customary lesions at the usual rate.

The majority of tumors (6 of 8 tumor lines) grown in chickens 18 months of age are not readily transferred to ducklings. However, some tumors (2 of the 8 lines) can be transferred to ducklings and in the course of several passages the tumor virus becomes adapted to the duck and loses totally or partially its power to induce tumors in chickens.

Tumors grown in chickens of intermediate age are more adaptable than are those grown in very young or old chickens. Out of a total of 15 tumors grown in 5 to 10 month old chickens, 9 became adapted to ducks following transfer to this species.

Reasons are given for considering that these changes in adaptability of chicken tumor virus are due to variations induced in the virus when it is grown in chickens of different ages and are not a consequence of the infection of the duck.—R. B.

Transplantability and Presence of Virus in Spontaneous Sarcomas and Fibromas of Chickens in Relation to the Age of the Tumor-Bearing Animal. DURAN-REYNALS, F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **6**:529-534. 1946.

Fourteen spontaneous chicken sarcomas have been grouped according to the age of the chicken bearing the growth. The presence of a causative virus, the transplantability of cells, and other properties of the tumor have been studied in each case. Of the 4 sarcomas found in chickens, ranging in age from 12 to 18 months, only 1 proved to be indefinitely transplantable and a causative virus was demonstrated by filtration. Of the 5 sarcomas found in chickens, ranging in age from 23 days to 18 weeks, none could be transplanted indefinitely although there are reasons to believe that 3 of them could have been so transplanted—1 being carried through 2 passages—while 2 others were carried through 1 passage. Of the 5 sarcomas found in chickens 5 to 10 months of age, 4 proved to be indefinitely transplantable with causative viruses demonstrated in their cell-free preparations, a circumstance probably true for the fifth sarcoma. Of the 7 fibromas, which occurred in chickens 8 to 30 months old, only one that was present in an 8 or 9 month old chicken, could be carried through 2 passages.

Although indefinite transplantability and subsequent presence of free virus were traits found only in sarcomas histologically classed as malignant, 7 other sarcomas with the same or a higher degree of histological malignancy failed entirely to show these features. On the other hand the fibroma carried through 2 passages was histologically non-malignant.

In summary, in our experience, a state of high malignancy in chickens was constantly associated with large, metastasizing sarcomas easily transplantable to chickens of several breeds and also to foreign species, namely, newborn ducks. These tumors were found most frequently arising in mature but not old chickens and also in some younger birds. Neoplastic viruses were demonstrated in all tumors that could be transplanted indefinitely.—Author's abstract.

A Study of Five Transplantable Chicken Sarcomas Induced by Viruses. DURAN-REYNALS, F., and SHRIGLEY, E. W. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **6**:535-544. 1946.

Five sarcomas spontaneously developing in chickens have been carried through this species in a number of passages by inoculation of cells, and they may be considered as being

capable of transplantation indefinitely. Four of them were found in chickens 5 to 10 months old; the fifth, in an 18 months old chicken. All showed metastases in the original host. A causative virus has been demonstrated in each of the tumors, and in 1 case in the original growth itself.

In 4 of the lines a hemorrhagic disease developed at the same time that free virus was demonstrated in cell-free preparations. In the fifth tumor, a slowly growing fibrosarcoma, the virus never induced hemorrhagic lesions although not infrequently metastases were present. Although the tumors can be classified as to certain common properties, each of them has typical features, which justifies the statement that no two of them are identical. Each of them raises different problems that are discussed.—Authors' abstract.

The Etiology of Fowl Tumors. Peacock, P. R. [Glasgow Royal Cancer Hosp., Glasgow, Scotland] *Cancer Research,* **6**:311-328. 1946.

Experience in transmitting chemically induced fowl sarcomas during the period 1935 to 1945 is described. A new sarcoma, G.R.C.H. 15, induced with 1,2,5,6-dibenzanthracene and propagated serially in pedigreed Brown Leghorn fowls of known genetic interrelationship, has, like earlier chemically induced sarcomas (G.R.C.H. 1 to 14), resisted all attempts to extract from it any infective causal virus.

Comparisons between sarcomas G.R.C.H. 1 to 15 and 3 virus sarcomas revealed slight but definite histological differences between the two groups. Analysis of 30 generations of serial passage of G.R.C.H. 15 sarcoma revealed a genetic basis for the resistance to tumor inoculation frequently encountered throughout these experiments. The seasonal variation in transmissibility previously reported for sarcomas G.R.C.H. 1 to 14 was also observed in the case of G.R.C.H. 15 sarcoma.

The classification of tumor extracts is discussed and a method of obtaining cell-free extracts by capillarity described. Typical results of irradiation of virus tumors and their extracts are described. Virus sarcomas show a tendency to recur in the margin of an irradiated area, differing in this respect from chemically induced fowl sarcomas and from transplantable mammalian sarcomas.

All the experimental results serve to emphasize the differences between our chemically induced fowl sarcomas and the virus sarcomas derived from fowls with spontaneous tumors or leukoses. Assertions that causal viruses in chemically induced fowl sarcomas have been demonstrated are analyzed and discussed. Only one such tumor (sarcoma No. 9 of McIntosh and Selbie) can be accepted as a virus-transmissible sarcoma derived from a tar sarcoma of apparently similar histology. This tumor seems to resemble closely the group of filtrable sarcomas of spontaneous origin.

A theoretical explanation of some apparently conflicting results is advanced, and the conclusion drawn that there is no reason to postulate a single virus or a group of closely allied viruses as the essential cause of all forms of cancer.—Author's summary.

Linkage and Crossing-Over Between Black Pigmentation and Susceptibility to Induced Fibrosarcoma in Mice. Strong, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] *Science,* **103**:554. 1946.

When mice are injected with methylcholanthrene for a number of generations germinal mutations appear among their descendants. One of these, a mutation to dominant black pigmentation from the ancestral brown stock, apparently involves also an increase in susceptibility to fibrosarcoma induced at the site of methylcholanthrene injection. All mice possessing the black gene, irrespective of strain of origin, show the increased susceptibility to induce fibrosarcoma. That the S^{fs} (susceptibility to induced fibrosarcoma) and black genes are in fact linked is demonstrated by the following experiment. Black mutants were crossed with the original brown ancestral stock (NHO descent). The F_1 generation (76 mice) showed the dominant black pigmentation and high susceptibility to induced fibrosarcoma. The backcross of these mice to the recessive brown stock produced 121 black and 114 brown mice. Of these the black backcross mice retained the high susceptibility of the black mutants and the F_1's, while the brown backcross showed the low susceptibility of the ancestral brown stock.

Some of the F_1 mice mentioned above appeared to have a greater resistance to induced fibrosarcoma than the majority of the F_1 group. This could be interpreted as due to loss of the hypothetical S^{fs} gene through crossing over. The correctness of this interpretation is indicated by the following experiment. One subline of black mutant derivatives, suspected of possessing low susceptibility to induced fibrosarcoma even though they were black, were outcrossed to the ancestral brown stock. The F_1's (255 mice) showed the low fibrosarcoma susceptibility of the brown stock. A new black subline derived from these resistant F_1 black mice continued to show the low susceptibility of the brown mice.—R. B.

Superficial "Burns" of Skin and Eyes from Scattered Cathode Rays. Robbins, L. L., Aub, J. C., Cope, O., Cogan, D. G., Langohr, J. L., Cloud, R. W., and Merrill, O. E. [Massachusetts Gen. Hosp., Huntington Memorial Hosp., Howe Lab. of Ophthalmology, and Massachusetts Inst. of Technology, Boston, Mass.] *Radiology,* **46**: 1-23. 1946.

During a procedure for localization of the focal spot of a 1,200 kv. electrostatic generator 6 men were exposed briefly to scattered cathode rays. They received an estimated 1,000 to 2,000 tissue roentgens. Only superficial layers of tissue were involved, with lesions showing the unusual characteristic of 3 distinct phases, appearing in 3 of the men. The first phase was prompt in appearance, superficial, and subsided in from 5 to 7 days. The effects of the second phase were more widespread, deeper and of longer duration. The third phase appeared about 4 weeks after exposure and was characterized by much more extensive involvement. It included a tertiary efflorescence which was widespread not only over new areas but also re-involved areas that seemed to be healing from the secondary reaction. Severe ocular signs were present in

the tertiary phase in one case. The account is given in full in order that a repetition of this experience in other laboratories might be prevented.—R. E. S.

The Possible Association Between Porphyrins and Cancer in Mice. Bittner, J. J., and Watson, C. J. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Cancer Research,* **6**:337-343. 1946.

The extraction of porphyrins from the harderian glands of virgin and breeding females of various inbred stocks of mice and F_1 hybrids would suggest that: There is no decrease in the porphyrin content of the harderian glands with increasing age. There is no relationship between the presence of the active milk agent and the porphyrins. There is no simple correlation between "inherited susceptibility" to mammary and lung cancer and the porphyrins. In every stock the breeding females showed higher porphyrin concentrations in the harderian glands than did the virgin females. Spayed females may show higher readings than virgin females of the same stock. The maximal concentrations in the various groups were attained at different ages. The apparent relationship between the porphyrins and mammary cancer, as described by others, may be due to a correlation of unknown nature between the porphyrins and the hormones. In virgin females this association may involve the "inherited hormonal influence."—Authors' summary.

The Water Content in the Epidermis of Mice Undergoing Carcinogenesis by Methylcholanthrene. Suntzeff, V., and Carruthers, C. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *Cancer Research,* **6**:574-577. 1946.

The water content of the epidermis of mice undergoing carcinogenesis by methylcholanthrene has been determined. The water content of normal untreated mouse epidermis was determined by drying the samples at 105° C. to constant weight and by extraction of the samples with toluene according to the Bidwell-Sterling procedure. Both methods gave nearly identical values. The water content of normal mouse epidermis was 60.3% whereas the average of the methylcholanthrene-treated epidermis was 66.5%, and moreover the water content of the latter was higher and constant throughout the "precancerous" stage. A squamous cell carcinoma, originally derived from mouse epidermis, contained an average water content of 81.7%, which is a much higher content than that of normal or hyperplastic epidermis.—Authors' abstract.

Apparent Prolongation of the Life Span of Rats by Intermittent Fasting. Carlson, A. J., and Hoelzel, F. [Univ. of Chicago, Chicago, Ill.] *J. Nutrition,* **31**:363-375. 1946.

Tests in which a group of 33 rats were allowed the same food *ad libitum* and 3 groups of 37, 37 and 30 rats each were fasted 1 day in 4, 3 and 2 days respectively, showed that after the age of 42 days the life span was increased by the intermittent fasting. The optimum amount of fasting appeared to be 1 day in 3 and this increased the life span of littermate males about 20% and littermate females about 15%. However, the pre-experimental condition of the individual rats was also found to be an important factor determining the life spans. No drastic retardation of growth was produced by the intermittent fasting but the development of mammary tumors was reduced in proportion to the amount of abstinence from food.—F. L. H.

Effect of Fresh Milk on the Production of Hepatic Tumors in Rats by Dimethylaminoazobenzene. Hoch-Ligeti, C. [Chester Beatty Research Inst., Royal Cancer Hosp., London, England] *Cancer Research,* **6**:563-573. 1946.

The addition of 10 cc. of fresh milk daily to a rice-carrot diet containing 0.06% of *p*-dimethylaminoazobenzene protected rats to a considerable extent from the development of liver tumors. In one group of 50 animals 2 incipient growths were found on the 410th and 460th days respectively; in a second group (30 animals) 1 tumor was found on the 161st day and another on the 262nd day. The addition of 6% casein to the rice-carrot diet with 0.06% of *p*-dimethylaminoazobenzene did not prevent the formation of tumors, 78% of the animals killed after the 90th day having had hepatic neoplasms.

The food intake of the animals on the diet containing milk decreased when *p*-dimethylaminoazobenzene was added. The body weights of the males were slightly lower than those of the controls receiving no carcinogen. However, no difference in the weights of females was observed. The food intake of the rats on rice and carrots, or rice, casein, and carrots with or without *p*-dimethylaminoazobenzene was about the same, and was about half as great as that of animals on a diet supplemented with milk. However, body growth was retarded or completely stopped no matter whether the carcinogen was included in the diet or not. Animals receiving the *p*-dimethylaminoazobenzene lost weight slightly. In all groups of rats receiving the chemical, the spleens were enlarged, and in those individuals that developed tumors, the spleens were smaller than in those without growths. The free HCl in the stomach generally disappeared in animals on diets producing tumors, but this was not always the case. Some rats on the rice-casein diet developed papillomatosis of the forestomach, the tendency to which being increased by the administration of azo dyes. Possible dietary influences on the development of primary liver cancer in some native peoples of Africa and Asia are discussed.—Author's summary.

Thiamine Deficiency and High Estrogen Findings in Uterine Cancer and in Menorrhagia. Ayre, J. E., and Bauld, W. A. G. [Royal Victoria Hosp., McGill Univ., Montreal, Canada] *Science,* **103**:441-445. 1946.

The endogenous estrogen levels (determined by degrees of cornification seen in vaginal and cervical smears) and the B-vitamin excretion levels were studied in 20 patients with proven uterine cancer. Ninety per cent of these exhibited low thiamine excretion coupled with abnormally high endogenous estrogen levels. Twenty per cent showed also a deficient excretion of riboflavin. Studies made on a similar group of menorrhagic patients showed the same correlation of thiamine deficiency and abnormally high estrogen level that was found among the cancer patients. In addition to the above studies, observations made on vaginal and cervical smears from 150 patients known to have cancer gave evidence of abnormally high estrogenic activity in more than two-thirds of them. Finally, a group of 180 patients with menorrhagia or

amenorrhea showed uniform clinical improvement (except in cases of proven inflammatory origin) following administration of B-vitamin complex. In some of these patients showing improvement there were striking decreases in degree of cornification as seen by vaginal and cervical smears.

The authors interpret these observations as follows: They think that nutritional deficiency, particularly in B-vitamins, may lead to impairment of the capacity of the liver to inactive estrogens which then accumulate in the body and exert a constant growth stimulus on estrogen-sensitive organs leading eventually to cancer.—R. B.

Effect of Rabbit Spleen Extract and Spleen Pulp on Mouse Carcinoma. GIERSCH, SR. C. [Inst. Divi Thomae, Salina, Kans.] *Stud. Inst. Divi Thomae,* **4**:9-14. 1945.

Male and female mice of the ABC strain were inoculated with 0.25 cc. of carcinoma 15091a suspension 1 week after the injection of rabbit spleen extract prepared by alternate freezing and thawing. This extract so prepared and administered may have increased the resistance of the host to carcinoma 15091a but did not protect against it.—E. B. B.

The Significance of Hyperemia around Tumor Implants. COMAN, D. R., and SHELDON, W. F. [Univ. of Pennsylvania Sch. of Med., Philadelphia, Pa.] *Am. J. Path.,* **22**: 821-825. 1946.

Hyperemia appeared within 18 hours after the implantation of fragments of tumor tissue into the subcutaneous tissue of mice and was present thereafter so long as the tumor grew. Homologous adult muscle tissue and heterologous tumor implants did not produce hyperemia, while homologous embryonic tissue excited hyperemia that faded as the embryonic tissue finally regressed. A tumor which grew briefly in C57 mice and then regressed, excited hyperemia during its short period of growth but failed to do so upon re-implantation in resistant hosts. The authors conclude that the hyperemia around mouse tumors is due to the presence of proliferating cells.—J. G. K.

Influence of Crown-Gall Bacterial Products, Crown-Gall Tissue Extracts, and Yeast Extract on Growth *in Vitro* of Excised Tobacco and Sunflower Tissue. HILDEBRANDT, A. C., RIKER, A. J., and DUGGAR, B. M. [Univ. of Wisconsin, Madison, Wisconsin] *Cancer Research,* **6**:368-377. 1946.

The effects of certain crown-gall bacterial metabolites, yeast extract, and crown-gall tissue extracts on the growth *in vitro* of sunflower and tobacco callus tissue have been studied. Supplements to the basic medium of fermented, cell-free, bacterial media of virulent and attenuated crown-gall cultures had little influence on the growth of these tissues at low concentrations, but there was a strongly inhibiting effect at the higher concentration. At increased concentrations lyophilized virulent and attenuated crown-gall bacteria cells were generally harmful to the growth of both tissues. The addition of autoclaved marigold crown-gall tissue extract, of unautoclaved tomato-gall extract, and of unautoclaved yeast extract to the basic medium generally stimulated growth at lower concentrations, but inhibited it at higher concentrations. Autoclaved Paris daisy crown-gall tissue extract at all concentrations was beneficial to sunflower tissue, but had either little or an inhibiting effect on tobacco tissue.—Authors' summary.

A Comparison of Two Levels of Roentgen and Neutron Irradiation of Normal and Lymphomatous Mice, Using Radiophosphorus as an Indicator of Cellular Activity. SCOTT, K. G. [Univ. of California, Berkeley, Calif.] *Radiology,* **46**:173-175. 1946.

In a series of experiments radiophosphorus was used to compare the effects of neutron and roentgen irradiation upon normal and lymphomatous tissue. It was found that neutrons and roentgen rays depress the phosphorus uptake of lymphomatous tissues in mice as measured by P^{32}. Neutrons appear to be roughly six times as effective as roentgen rays. By the use of the technic used in these experiments another method of study of the effects of radiation on tissues is available.—R. E. S.

The Effect of Tocopherols in Preventing Gastric Ulcers in Rats. JENSEN, J. L. [Research Labs., Distillation Products, Inc., Rochester, N. Y.] *Science,* **103**:586-587. 1946.

Rats maintained on a vitamin A deficient diet developed gastric ulcers. When 0.5 to 5 mgm. of *d,α*-tocopherol was given as a daily supplement to the vitamin A deficient diet, the ulcers were prevented completely from appearing.—R. B.

Radioactive Phosphorus as a Therapeutic Agent. A Review of the Literature and Analysis of the Results of Treatment of 155 Patients with Various Blood Dyscrasias, Lymphomas, and Other Malignant Neoplastic Diseases. REINHARD, E. H., MOORE, C. V., BIERBAUM, O. S., and MOORE, S. *With an Appendix:* **The Assay of Radioactive Phosphorus.** KAMEN, M. D. [Washington Univ. Sch. Med., St. Louis, Mo.] *J. Lab. and Clin. Med.,* **31**:107-218. 1946.

The rationale for the therapeutic use of radioactive phosphorus is presented. Probably the best therapeutic agent available for polycythemia vera is P^{32} which induces satisfactory clinical and hematologic remissions lasting up to more than two years. Radioactive phosphorus is effective in producing hematologic remissions in chronic myelogenous leukemia but has little effect on the clinical course of the acute or subacute disease. Treatment of chronic lymphatic leukemia with P^{32} is probably as satisfactory as, but no better than, x-radiation in relieving symptoms and prolonging life. X-radiation is a more effective and a less dangerous form of therapy than P^{32} in the treatment of Hodgkin's disease, multiple myeloma, lymphosarcoma, reticulum cell sarcoma and giant follicular lymphoblastoma, because of the unfavorable influence of P^{32} on marrow. Radioactive phosphorus is probably not satisfactory for the treatment of acute lymphatic leukemia, monocytic leukemia, lymphoepithelioma, malignant melanoma, carcinoma of the gall bladder, carcinoma of the breast, Ewing's tumor, mycosis fungoides or xanthomatosis. Since x-radiation is usually more effective in bringing about a rapid reduction in the size of the spleen or lymph nodes, it can be used to supplement P^{32}.

Radioactive phosphorus has a profound effect on bone marrow and severe leukopenia, thrombocytopenia and anemia may occur as complications. Therapy must be individualized to a high degree. Of importance in cal-

culating dosage is the assay of phosphorus salt activity which varies with different methods.—C. R. N.

Influence of Carbamic Esters (Urethanes) on Experimental Animal Tumours. Haddow, A., and Sexton, W. A. [Chester Beatty Res. Inst., Royal Cancer Hosp. (Free), London, England, and Imperial Chemical Industries Ltd., Blackley, Manchester, England] *Nature, London,* **157**: 500-503. 1946.

In an investigation of the action of carbamic esters upon the growth of experimental tumors, phenylurethane and isopropyl phenylcarbamate were found to cause a slight but significant retardation of the growth of spontaneous mammary cancer in the mouse and of the Walker tumor in the rat. The response persisted only during administration and passed off rapidly when the drug was withdrawn. There was also observed, following the administration of phenylurethane, a transient increase of the mitotic count in the crypts of Lieberkühn in the mouse gut, an effect of interest in view of statements by other authors that phenylurethane produces mitotic effects similar to those described for colchicine. It was then found that the same effects are also produced by ethylcarbamate (urethane) itself, and that this substance also brings about a profound modification in the histological structure of the Walker sarcoma, the characteristic cellular structure giving place to a more fibrous architecture, with spindle-cells and an abundant stroma.

The nature of these effects and the fact that they might be brought about by a known substance as simple and as readily available as urethane, suggested the advisability of testing the action of these drugs on inoperable cancer of the human subject (see following abstract). The present paper discusses possible modes of action of urethane with reference to the relation between narcosis, inhibition of cell division, inhibition of dehydrogenases, and a suggestion that urethane might conceivably act by competing with some natural amine involved in the biosynthesis of nucleotides.—A. H.

Leukaemia Treated with Urethane. Compared with Deep X-Ray Therapy. Paterson, E., Thomas, I. A., Haddow, A., and Watkinson, J. M. [Christie Hosp., and Holt Radium Inst., Manchester, and Royal Cancer Hosp. (Free), London, England] *Lancet,* **1**:677-682. 1946.

Following experimental work by Haddow and Sexton on the growth-inhibitory action of urethane on animal tumors, it was decided in 1943 to test the drug in advanced cases of malignant disease in man. In some of these cases a fall in the leukocyte count was noticed and this suggested the trial of urethane in patients with leukemia. Urethane has now been used in 32 cases (19 myeloid and 13 lymphatic) observed over periods ranging from 5 weeks to 11 months. The effects produced as seen in the most favorable cases, were a fall in the total white cell count to normal limits, a tendency for the differential count to approach a more normal pattern, a diminution in the size of the spleen and of enlarged lymph nodes, and a rise in hemoglobin level. These results are remarkably similar to those obtained by standard methods of deep x-ray therapy.

There is no indication that permanent benefit may result from the use of urethane in either myeloid or lymphatic leukemia, for relapses take place and immature cells may reappear in the blood. The cases are too recent to enable any statement to be made about the effect of treatment on length of life. It can, however, be stated that the palliative effect is in many cases very great. Urethane has been administered also in 13 cases of advanced carcinoma of the breast and in 11 cases of other types of malignant disease. A moderate leukopenia was observed in 9 of these. In 3 of the breast cases and 4 of the miscellaneous group there was a temporary diminution in the size of the lesions.—A. H.

Some Observations on X-Ray Treatment Cones. Eddy, C. E., and Stevens, D. J. [Univ. of Melbourne, Melbourne, Australia] *Radiology,* **46**:176-183. 1946.

A study of x-ray treatment cones indicates that many cones have disadvantages and inaccuracies which should be recognized and corrected. The authors believe a field defined by a diaphragm at the top of the cone is more satisfactory than one which depends on the radiopaque walls of the cone. Photographic examination of a cone field will indicate the existence of any physical defects or leakage, if such are present. The field produced by extremely small cones is often non-uniform and has a lower dosage than would be expected. It is felt that larger cones with the skin areas limited by a lead screen with suitable aperture are best. Intracavitary cones should be carefully aligned. Hard rubber intravaginal specula should be carefully examined from the physical point of view before using.—R. E. S.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

Heredity

Tumors and Short-Toe—A Dihybrid Pedigree. A Family History Showing the Inheritance of Hemangioma and Metatarsus Atavicus. Beers, C. V., and Clark, L. A. [Univ. of Southern California, Los Angeles, Calif.] *J. Hered.,* **33**:366-368. 1942.

This report concerns the inheritance for 3 generations of hemangioma and a short first toe. Hemangioma is inherited as an autosomal dominant from a presumably homozygous man, since in this family it (a) does not skip a generation, and (b) each affected individual has a parent with the character. One man has 5 children, all affected. Short first toe is inherited as an autosomal dominant from a heterozygous woman. There is no clear evidence of linkage between hemangioma and short first toe.—G. W. W.

The Inheritance of Nevi. Denaro, S. J. [Radcliff Coll., Cambridge, Mass.] *J. Hered.,* **35**:215-218. 1944.

New evidence is presented concerning the inheritance of birthmarks or nevi. The results of these studies

strengthen the indication that the inheritance involves dominant or partly dominant genes. These genes may be entirely suppressed in certain generations of a pedigree. The characteristic localization of the nevi in different families suggests that different genes are involved in different families or that different complexes of modifying factors are present.—G. W. W.

Race, Stock and Environment in Human Cancer. Peller, S. [New York, N. Y.] *J. Hered.*, **35**:175-181. 1944.

Peller reviews his statistical studies on the relation between the incidence and mortality of skin and lip cancer and that of "internal organs" and compares them with some of the findings of the U. S. Public Health Service cancer morbidity surveys. These surveys indicated that, among white persons in the South, the annual prevalence (all cases under medical supervision during a year) of skin and lip cancer was 3 or 4 times as great as in the North. In contrast, cancer of the "digestive and respiratory organs" was about one-third less frequent in the South. On the other hand, the low prevalence of skin and lip cancer among Negroes was not accompanied by a corresponding increase in most other sites of cancer (except that of the uterus). These latter data are considered inaccurate. The author reiterates his view that the induction of skin cancer decreases the likelihood of developing certain forms of internal cancer. He suggests that the experiment be made in members of families known to be highly susceptible to breast or gastrointestinal tract cancer.—M. L. L.

Diagnosis—General

Diagnostic Value of Exfoliated Cells from Cancerous Tissues. Papanicolaou, G. N. [Cornell Univ. Med. Coll., New York, N. Y.] *J.A.M.A.*, **131**:372-378. 1946.

The diagnostic value of the smear test is based on the exfoliation of malignant neoplasms having a free surface, cells becoming detached singly or in clusters. Small fragments of tissue in the form of cell clusters are diagnostic aids as they retain more of the structural characteristics of the neoplasm than the individual cell. Here the size and form of the nuclei are most significant. The form of the cancer cell depends on the prevailing type of cancer. Whereas the nucleus tends to show uniform and comparable changes in all types of malignant neoplasms, the cell as a whole largely retains its individuality and its specific form. The technic of collecting, preparing and staining the smears differs only slightly with various specimens. The method is adaptable to a study of vaginal, endocervical and endometrial fluids, sputum, urine, gastric aspirations, and thoracic and peritoneal fluids with a high degree of diagnostic accuracy.—M. E. H.

Cervical Cytology Tests in Cancer Diagnosis: Glycerine Technique for Mailing. Ayre, J. E., and Dakin, E. [Royal Victoria Hosp., McGill Univ., Montreal, Canada] *Canadian M.A.J.*, **54**:489-491. 1946.

Smears of cervical secretions fixed in formalin, or ether and alcohol and dried before staining were found on study not to have retained clear nuclear definition. On the other hand smears treated with a drop of glycerine after removal from the fixative and not allowed to dry, retained on staining the more delicate morphological features which are necessary to permit accuracy in cancer diagnosis.—M. E. H.

Sialography. Beyer, T. E. [Denver, Colo.] *Rocky Mt. M. J.*, **43**:210-216. 1946.

Sialography, roentgen visualization of the salivary glands and ducts after the injection of radiopaque substance into their respective ducts, marks a distinct advance in diagnosis. Contraindicated in acute parotitis whether infectious or surgical, its greatest field of usefulness would seem to rest in determining the correct operative procedure to be employed in tumors of the parotid. In tumors of the submaxillary region, sialography is of very limited value.—M. E. H.

Radiation Therapy Conference. The Radiotherapeutic Test in the Differential Diagnosis of Mediastinal Pathology. Cantril, S. T., and Buschke, F., Editors. [Tumor Inst., Swedish Hosp., Seattle, Wash.] *West. J. Surg.*, **54**:166-169. 1946.

The response of a mediastinal lesion to x-ray therapy has long been used as an important adjunct in the differential diagnosis of mediastinal pathology. In general, lymphosarcomas and mediastinal preleukemic adenopathies respond very rapidly and the response is demonstrable clinically and radiologically usually in a few days. In Hodgkin's disease, the dose producing clinical and radiographic regression is definitely higher and the regression much slower. This observation is of importance since the treatment for these different types of lesions should be conducted in quite a different manner. For clinical purposes, an attempt at careful differentiation among these three types of lesions is essential.—M. E. H.

Chorionic Gonadotropin in the Diagnosis of Testicular Tumors. Brewer, J. I. [Chicago Pathological Society, Regular Meeting, March 11, 1946] *Proc. Inst. Med. Chicago*, **16**:170. 1946.

Two gonadotropins, chorionic and castrate types, may be present in the urine of some patients with primary testicular tumors. Only chorionic tissue is known to produce the chorionic gonadotropin. When the latter is present in the urine it indicates that chorionic tissue comprises a part, at least, of the testicular tumor or its metastases.—M. E. H.

Diagnosis and Treatment of Rectosigmoid Malignancy. Holehan, M. W. [Univ. of Tennessee, Memphis, Tenn.] *J. Tennessee M. A.*, **38**:295-300. 1945.

A general discussion urging careful examination of all patients presenting any change in bowel habits is given. Features are enumerated that may be useful in distinguishing carcinoma from polyps, adenomas, lymphosarcoma and lymphopathia venereum. Greater detail is devoted to the operative procedure and care of the patient. —E. E. S.

Therapy—General

The Treatment of Advanced and Inoperable Cancer. Howes, W. E., and Shapiro, A. L. [Brooklyn Cancer Inst., Brooklyn, N. Y.] *Surgery*, **18**:207-228. 1945.

The symptoms and problems peculiar to each of 3 large groups of advanced cancer are outlined, and the palliative measures indicated in each are discussed. The authors delineate these groups as: (1) those patients with

head and neck cancers, (2) the abdomino-thoracic group, and (3) the sarcomas. The data on 42 representative cases of advanced and recurrent carcinoma are tabulated, listing all types of therapy utilized and the results obtained. Of this and other groups the authors believe that 70% of the patients obtain much benefit from palliative measures, and 5% are actually salvaged.—W. A. B.

X-Ray Therapy of the Heart in a Patient with Leukemia, Heart Block and Hypertension. Blotner, H., and Sosman, M. C. [Peter Bent Brigham and Beth Israel Hosps., Boston, Mass.] *New England J. Med.*, **230**:793-796. 1944.

Report of a case in which x-ray therapy caused a heart block to disappear by its effect on a possible leukemic infiltration that involved the conduction mechanism of the heart.—C. W.

The Hormonal Treatment of Prostatic Malignancy. Deming, C. L. [Yale Univ. Sch. of Med., New Haven, Conn.] *Bull. New York Acad. Med.*, **22**:88-101. 1946.

A general review of the subject is given in which the author concludes that castration and stilbestrol treatment are palliative and not curative measures in cancer of the prostate. Castration and stilbestrol produce the same results; pain is completely relieved temporarily in at least 50% of the cases and there is a temporary return to normal or comfortable micturition in 50 to 75% of cases. Regression of secondary lesions in the lungs and femur has been reported, but neither castration nor estrogenic therapy prevents the development of new metastases. The only complete control of cancer of the prostate in the author's clinic has been obtained by radical surgical excision early in the development of the disease.—M. T.

Prophylaxis Against Cervical Malignancy. Kennedy, J. W. [Philadelphia, Pa.] *Virginia M. Monthly*, **73**:51-52. 1946.

The paper deals with cervical malignancy following neglected injuries incident to child-bearing.—M. E. H.

The Care of the Cancer Patient. Reimann, S. P. [Lankenau Hosp., Philadelphia, Pa.] *Pennsylvania M. J.*, **48**: 450-454. 1945.

A general discussion on the care of the cancer patient in which a plea is made for detailed care of the patient in the home. The author believes that in many instances too much attention is paid to the cancer itself and not enough to the patient.—J. L. M.

Effects of Therapeutic Doses of X-Ray and Radium on the Human Uterus and Uterine Carcinomas. Presidential Address. Sheehan, J. F. [Chicago Pathological Society, Regular Meeting, Dec. 10, 1945] *Proc. Inst. Med. Chicago*, **16**:78-81. 1946.

Of the cases of squamous cell carcinomas presented, 4 ultimately disappeared, 3 were regressing but 3 recurred as early as 2 weeks after cessation of therapy. Some adenocarcinomas responded to radiation and even occasionally are destroyed. The changes that were considered to be direct effects of radiation on the uterus itself, were necrotic plaques at or near the internal os, alterations in the myometrium and cervix, and certain arterial changes.—M. E. H.

Subtotal Hysterectomy for Cancer of the Cervix and Uterus. Radiation Therapy Conference. Cantril, S. T., and Buschke, F., Editors. [Tumor Inst. of the Swedish Hosp., Seattle, Wash.] *West. J. Surgery*, **54**:211-212. 1946.

Lack of appreciation of the common association of cancer of the cervix and of the uterine body with fibromyomas has resulted in subtotal hysterectomy when either more radical surgery or radium therapy or both procedures have been indicated. More precise preoperative diagnosis including curettage should be demanded before subtotal hysterectomy is attempted.—M. E. H.

The Betatron and Its Medical Application. Kerst, D. W. [Chicago Roentgen Society, Regular Meeting, March 14, 1946] *Proc. Inst. Med. Chicago*, **16**:202. 1946.

The pioneer work on the nature of the biological reactions produced by betatron radiation and on the biological value of the r unit of x-radiation from a 20 million volt betatron shows that with proper caution and practice the radiologist should be able to administer a dose of betatron radiation and obtain biological reactions that are fundamentally no different from those observed with a 200 kilo volt x-ray machine, but which are different in special distribution.—M. E. H.

Prostatic Cancer Treated by Orchiectomy: The Five Year Results. Huggins, C. [Univ. of Chicago, Chicago, Ill.] *J. A. M. A.*, **131**:576-581. 1946.

The results of treatment by orchiectomy of 20 patients with prostatic carcinoma ranging in age from 54 to 80 years, reveal that 4 have succumbed in less than a year, 2 showing no obvious benefit from the procedure. Seven died during the first 2 years following the operation and 4 additional patients between 36 and 63 months. Five of the 20 patients have survived more than 5 years. No sign of the disease can be detected in 4 of these; in the fifth there is a tumor the size of a hen's egg in the region of the seminal vesicle. All 5 patients had widespread metastases to bone and elevated serum phosphatase at the time of orchiectomy. In each case, after 5 years, the enzyme levels are within normal range and the bones show equivocal or no evidence of metastasis.—M. E. H.

Excision of Superior Maxilla and Ethmoid Labyrinth. Passe, E. R. G., [London, England] *Arch. Otolaryng.*, **43**:379-392. 1946.

Case report. A transoral approach for excising the superior maxilla in its entirety is described. This operation is applicable in cases of cancer and certain chronic diseases of this region.—C. R. N.

Clinical Results in Patients Treated by Intranasal Ethmoidectomy. Dixon, F. W. [Cleveland, Ohio] *Arch. Otolaryng.*, **43**:59-62. 1946.

An intranasal ethmoidectomy with the subsequent ventilation and drainage of other sinuses offers the best solution to restore to a nearly normal condition a nasal cavity filled with polyps. Case reports.—C. R. N.

Skin and Subcutaneous Tissues

Generalized Erythrodermia (? Malignant Reticullosis). Levy, H. *Proc. Roy. Soc. Med.*, **39**:300-302. 1946.

Description of a case.—E. L. K.

Melanoma of the Skin with Intraocular and Orbital Metastases. Report of a Case. Lisa, J. R., and Givner, I. [City Hosp., New York, N. Y.] *Arch. Ophth.*, **34**:422-424. 1945.

Report of a case of melanoma of the skin, with widespread metastases.—E. C. R.

Kaposi's Idiopathic Sarcoma. Wigley, J. E. M. *Proc. Roy. Soc. Med.*, **39**:227. 1946.

The majority of cases seen in London have been in males of Central European Jewish origin. This case, however, was in a woman without Jewish ancestry.—E. L. K.

Primary Healing Following Excision of a Cyst, Teratoma. Cooper, C. N. [U.S.N.R.] *U. S. Nav. M. Bull.*, **45**:474-478. 1945.

Description of surgical technic employed to obtain primary healing following removal of pilonidal cyst.—C. W.

Metastatic Carcinoma to the Choroid Arising from the Lip. Goodsitt, E. [East Cleveland, Ohio] *Am. J. Ophth.*, **28**:1256-1258. 1945.

Report of a case of carcinoma of the choroid which was metastatic from a squamous cell carcinoma of the upper lip.—E. C. R.

Cancer of the Lip. Editorial. *Pennsylvania M. J.*, **48**: 587. 1945.

The point is made that an alert anticipatory observation with adequate surgical or radiologic procedures is all that is required to make cancer of the lip a rare rather than a common disease.—J. L. M.

Nervous System

Divergence Paralysis Associated with Tumor of the Brain. Savitsky, N., and Madonick, M. J. [Montefiore Hosp., and Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Arch. Neurol. & Psychiat.*, **55**:232-235. 1946.

The authors report four additional, proved cases of divergence paralysis associated with tumors of the posterior fossa; 2 were cerebellar tumors and 2 acoustic neuromas. The divergence paralysis was the result of involvement in some way of the divergence center in the brain stem. It disappeared in the 3 cases in which the tumor was successfully removed.—M. E. H.

Cerebellar Medulloblastoma. Manlove, C. H. [Portland, Ore.] *Northwest Med.*, **45**:339-343. 1946.

Report of a case in which a tumor, arising as a small nodule in the roof of the fourth ventricle, extended into the subarachnoid space of the brain and spinal cord so extensively that differentiation from sarcomatosis of the leptomeninges could only be made after histological examination of specially stained sections.—H. S. K.

Paralysis of Divergence Due to Cerebellar Tumor. Lippmann, O. [Austin, Tex.] *Arch. Ophth.*, **31**:299-301. 1944.

There is no generally accepted opinion as to the mechanism of divergence in human eyes, and this report deals with a discussion of this subject. Paralysis of divergence was exhibited by a 20 year old man with a cerebellar tumor. The type of tumor present was difficult to classify. Operation and autopsy findings are included.—E. C. R.

Neurofibroma of the Cauda Equina: Report of a Case. Pomeranz, R. [A.U.S.] *Radiology*, **44**:588-592. 1945.

A case report of a small encapsulated intradural lesion recognized preoperatively by radiographic and myelographic studies is presented.—R. E. S.

Tuberculoid Reaction in a Retroperitoneal Neurocytoma. Hartz, P. H., and Van Der Sar, A. [Pub. Health Service, Curaçao, N. W. I.] *Am. J. Clin. Path.*, **15**:473-477. 1945.

Detailed report of a case in a 14 month old negro child in whom tuberculosis could not be demonstrated clinically.—J. G. K.

Lesions of the Spinal Epidural Space Producing Cord Compression. Shenkin, H. A., Horn, R. C., and Grant, F. C. [Hosp. of Univ. of Pennsylvania Sch. of Med., Philadelphia, Pa.] *Arch. Surg.*, **51**:125-146. 1945.

A series of 54 cases is reported; all these patients underwent laminectomy for relief of cord compression. The types of lesions found and number of each were: 9 metastatic carcinomas, 7 myelomas, 6 neuroblastomas, 3 giant cell tumors of bone, 10 miscellaneous epidural tumors, 2 spinal extradural cysts, 3 epidural hemorrhages, and 14 with epidural infectious processes. In the older age groups myeloma and metastatic carcinoma were most common. Three of the neuroblastomas and all 3 giant cell tumors of bone occurred in subjects less than 20 years old. Tuberculous granuloma, extradural cysts and epidural abscesses were also among the lesions encountered in the first 2 decades of life.—W. A. B.

Eye

Bowen's Disease of the Cornea. Weskamp, C. [Univ. Nacional del Litoral, Rosario, Argentina] *Arch. Ophth.*, **31**:310-315. 1944.

Bowen's disease is regarded by some dermatologists as a precancerous dyskeratosis, by others as cancerous from the onset. In the case reported here, a superficial corneal lesion was the only manifestation of the disease: neither the skin nor the mucous membrane was involved. Excision of the lesion left the patient with a clear cornea and improved vision. Histologic examination of the excised specimen revealed that the lesion was an epithelioma. Large, clear cells with irregular nuclei and cells of the so-called dyskeratotic type, exactly as they appear in Bowen's disease of the skin, were demonstrated.—M. H. P.

Tuberous Sclerosis Associated with Tumor of the Optic Disk (Phacoma). Glicklich, E. A., Schultz, A., and Benjamin, J. E. [A. U. S.] *Arch. Ophth.*, **32**:60-62. 1944.

This is the report of a case of tuberous sclerosis having the characteristic syndrome of adenoma sebaceum, epilepsy, and mental deficiency. In addition, a tumor of the optic disk (phacoma) was present.—E. C. R.

Leiomyoma of the Iris. A Case Report. Davis, W. T., Sheppard, E., and Romejko, W. J. [George Washington Univ. Med. Sch., Washington, D. C.] *Am. J. Ophth.*, **27**:467-469. 1944.

A leiomyoma of the iris, present since childhood, was removed, together with adjacent iris tissue, from the eye

of a 27 year old woman. This tumor grows slowly, causes no symptoms, and does not invade the surrounding structures. For surgical therapy, therefore, excision should be considered before enucleation. It is impossible clinically, however, to differentiate leiomyoma from malignant tumors of the iris. A review of the literature, bibliography, photographs, and a photomicrograph accompany this report.—M. H. P.

Occurrence of Glioma of Retina and Brain in Collateral Lines in Same Family. Genetics of Glioma. Rados, A., [Beth-Israel Hosp., Newark, N. J.] *Arch. Ophth.*, **35**:1-12. 1946.

The hereditary basis of malignant growths, especially that of retinoblastoma, is discussed. A case of glioma of the brain and one of glioma of the retina, occurring in collateral lines are reported.—E. C. R.

Ophthalmologic Features of Intracranial Chordoma and Allied Tumors of the Clivus. Givner, I. [New York, N. Y.] *Arch. Ophth.*, **33**:397-403. 1945.

A chordoma is a neoplasm arising from embryonic rests of the chorda dorsalis, a specific embryonal tissue about which the spinal column develops. Diplopia and visual disturbances occur in over one third of the cases, and they are thus of great interest to the ophthalmologist. In this paper interest is directed only to the spheno-occipital tumors and not to growths in the sacrococcygeal or vertebral area or in the region of the jaw. Two cases are recorded one of which is reported in detail with autopsy findings.—E. C. R.

Errors in Diagnosis of Intraocular Tumors. Bruner, W. E. [Cleveland, Ohio] *Am. J. Ophth.*, **28**:297-302. 1945.

A general discussion based on 7 cases that illustrate well the difficulties and dangers in the diagnosis of intraocular tumors.—E. C. R.

Myxoma of the Lower Eyelid. Town, A. E. [(MC) U.S.N.R., Bethesda, Md.] *Am. J. Ophth.*, **28**:68-69. 1945.

Report of a case.—E. C. R.

Bilateral Teratoid Tumor of the Limbus. Rosen, E. [(M.C.) A.U.S.] *Arch. Ophth.*, **32**:120-122. 1944.

Report of a case observed in an apparently healthy young man 25 years old. The condition caused poor vision in one eye, but vision in the other eye was unaffected. The diagnosis was confirmed by biopsy.—E. C. R.

The Management of Epibulbar Malignancy. Fry, W. E. [Univ. of Pennsylvania, Philadelphia, Pa.] *Pennsylvania M. J.*, **48**:694-696. 1945.

The more frequent lesions that occur at the limbus are described, and their treatment given. Two case reports are presented showing (1) that sarcoma of the limbus may remain localized at the limbus for a period of years without extensive involvement of the cornea, without intraocular extension, and without distant metastasis, and (2) that epithelioma of the limbus may extend into the cornea at an early stage.—J. L. M.

Recurrent Juvenile Papilloma of the Conjunctiva. Walker, J. D. [Houston, Tex.] *Am. J. Ophth.*, **28**:751-754. 1945.

A report of a case is presented in which a benign, but rapidly spreading papilloma of the conjunctiva was situated at the inner canthus of the right eye and involved the caruncle and the mucous membrane of the lower lacrimal canaliculus. The tumor was removed on 14 separate occasions by dissection and treated by x-ray and radium before being finally controlled.—E. C. R.

Diffuse Neurofibromatosis (Von Recklinghausen's Disease) Involving the Bulbar Conjunctiva. Allende, F. P. [Italian Hosp., Sante Fé, Argentina] *Arch. Opth.*, **33**: 110-115. 1945.

A case of a large neurofibroma involving the bulbar conjunctiva of the right eye is presented. The author states that only 3 other instances have been recorded in which tumors of this type were found in this location. The patient, a woman 18, had in addition similar lesions of the skeletal system and skin, bodily asymmetry, and intracranial involvement.—E. C. R.

Treatment of Bilateral Retinoblastoma (Retinal Glioma) Surgically and by Irradiation. Report on Progress. Martin, H., and Reese, A. B. [Memorial Hosp., New York, N. Y.] *Arch. Ophth.*, **33**:429-439. 1945.

Retinoblastoma is a congenital, radiosensitive tumor that is usually bilateral. Removal of the eye that is more involved is advised, followed by irradiation of the remaining eye by a special technic, which is described. Results of treatment of 14 additional patients are recorded, making a total of 24 cases that have received this type of therapy. In about half the cases, varying degrees of useful vision have been preserved.—E. C. R.

Breast

Breast Cancer and "Paget's Disease of the Breast." Costello, C. J. [Barnard Free Skin and Cancer Hosp., Washington Univ. Sch. of Med., St. Louis, Mo.] *Arch. Surg.*, **51**:262-278. 1945.

The lesion known as "Paget's disease of the breast" begins as an intractable eczema of the nipple or areola. In the author's series of 29 patients, all were over 30 years old, but in some the cutaneous lesion had been present for very long periods—in one for 40 years. All these lesions when subjected to biopsy showed the typical Paget cells, which the author prefers to call large, neoplastic cells, with hyperchromatic nuclei, showing occasional mitotic figures. This cell is characteristically surrounded by a clear space, with no intercellular bridges between it and the prickle cells, a fact which differentiates Paget's from Bowen's disease, in which the large intra-epidermal neoplastic cell is connected with epidermal cells by intercellular bridges. It is recommended that the terms "Paget's disease of the breast" and "extramammary Paget's disease" be superseded by "secondary epidermal carcinoma," which explains the nature of the lesion and may facilitate earlier and more rational therapy.—W. A. B.

Osseous, Cartilaginous, and Mixed Tumors of the Human Breast. Rattino, A., and Willson, K. [New York, N. Y.] *Arch. Surg.*, 50:184-193. 1945.

This is a review of the literature on a group of rare tumors of the breast. Included are enchondroma, chondrosarcoma, osteosarcoma, osteoid sarcoma or giant cell sarcoma, mixed tumor, teratoma and chondrolipoma, ossifying, chondrifying and giant-cell tumor associated with

carcinoma, and carcinosarcoma. All these tumors are characterized by bone formation, cartilage, fibrous tissue, and pleomorphic cells, and are essentially malignant.—W. A. B.

Metastatic Carcinoma of the Choroid. Johnston, K. B. [Montreal, Canada] *Canad. M. A. J.*, **54**:46-48. 1946.

The 4 cases reported showed the lesions to be secondary to carcinoma of the breast, which is the most common site of the primary growth.—M. E. H.

Female Genital Tract

Another Case of Arrhenoblastoma. Curtis, A. H. [Chicago Gynecological Society, Regular Meeting Nov. 16, 1945] *Proc. Inst. Med. Chicago*, **16**:76. 1946.

Microscopic examination identified the ovarian tumors as arrhenoblastoma of the intermediate group.—M. E. H.

Origin of Adrenal-Like Tumor of the Ovary (Hypernephroma of Ovary, Adrenal Tumor of Ovary, Masculinovoblastoma, Luteoma, Luteinoma). Curtis, A. H. [Chicago Gynecological Society, Regular Meeting Nov. 16, 1945] *Proc. Inst. Med. Chicago*, **16**:76-77. 1946.

Only 15 authentic cases have been reported. All have presented symptoms of masculinization; in addition some patients had glycosuria, polycythemia and hypertension. Another case is reported and the genesis and nature of the growth is discussed.—M. E. H.

Endometriosis. Low, D. M. [Toronto, Canada] *Canad. M. A. J.*, **53**:49-52. 1945.

This is a report of 249 patients with endometriosis of various organs, treated surgically. Ninety of the patients were subjected to conservative operation for the preservation of the reproductive function. Of these, 39 failed to benefit; 26 required second operations. In subsequent successful pregnancies, 9 children were saved, a result that the author believes is ample justification for continued attempts at truly conservative surgery in suitable cases.—M. E. H.

Endometriosis Causing Intestinal Obstruction. Wood, O. T., Deikert, I., and Kain, T. [Philadelphia, Pa.] *J. A. M. A.*, **130**:341-343. 1946.

A case report, recorded because of the short duration of the symptoms and because it represented an instance of acute complete obstruction, which necessitated resection of a part of the ileum.—M. E. H.

Leiomyosarcoma of the Uterus with Metastasis to the Femur. Brooke, W. S., and Thomason, J. R. [Univ. of Utah Sch. of Med., Salt Lake City, Utah] *Arch. Surg.*, **51**:120-124. 1945.

This is the fourth reported case in the literature of bone involvement by leiomyosarcoma of the uterus. The patient, a woman of 43 years, had had a sub-total hysterectomy at the age of 38, following a presumptive diagnosis of uterine myomas. The pathologist reported malignant degeneration (myosarcoma) of a submucous fibroma. Four years later she began to have pain in the left knee and hip, and x-rays showed erosion of the neck of the femur. She did not return for treatment until a fracture of the neck of the femur occurred. Biopsy of the femur at the fracture site showed extension of the uterine leiomyosarcoma, a diagnosis later confirmed at autopsy.—W. A. B.

Chorionepithelioma. Tew, W. P. [Univ. of Western Ontario, London, Ontario, Canada] *Canadian M. A. J.*, **54**: 469-472. 1946.

Four cases of chorionepithelioma are reported; 1 of the 4 was associated with a hydatidiform mole. Two of the patients were alive and well 7 years after operation and 2 have died. In one case the lesion became manifest following a full term labor and in 3 others after an early pregnancy or an abortion. The importance of a thorough investigation of any unusual bleeding following a full term delivery or an abortion is emphasized as well as the early use of diagnostic curettage and the Friedman test. The Friedman test, when strongly positive, is most dependable for diagnosis.—M. E. H.

Chorio-epithelioma with Herpes Gestationis. Report of a Case. Whitfield, J. M., Smith, L., and Manson, R. C. [Richmond, Va.] *Virginia M. Monthly*, **73**:257-262. 1946.

This case is reported as the second in the literature which combines the unusual conditions of chorionepithelioma and herpes gestationis.—M. E. H.

Male Genital Tract

Papillary Carcinoma Arising from Rete Testis. Feek, J. D., and Hunter, W. C. [Portland, Ore.] *Arch. Path.*, **40**:399-402. 1945

Case report.—J. G. K.

Cancer of the Prostate in Connecticut. Griswold, M. H. [Chief, Division of Cancer Research, Connecticut State Department of Health, Hartford, Conn.] *Connecticut M. J.*, **10**:106-109. 1946.

Refinements in diagnosis and more searching physical examinations of apparently simple hypertrophy have brought to recognition an increasing number of cancers of the prostate. The introduction of castration and the use of estrogenic hormones has justified the belief that something can be done for patients therapeutically. This initial report will act as a base line against which new methods of treatment may be measured.—M. E. H.

Urinary System

Skeletal and Pulmonary Metastases from Cancer of the Kidney, Prostate and Bladder. Freid, J. R. [New York, N. Y.] *Am. J. Roentgenol.*, **55**:153-164. 1946.

Skeletal and pulmonary metastases were investigated, either by roentgen or postmortem examinations, in 203 patients who died from cancer of the kidney, prostate and bladder. Of 87 patients with cancer of the kidney, 39 cases, or 45%, revealed skeletal metastases. In 47 instances, or 54%, pulmonary, pleural or mediastinal metastases were also present. Sixty patients with cancer of the prostate were investigated. In 35 cases, or 58%, skeletal metastases were found. Pulmonary, pleural or mediastinal involvement was discovered in 26 cases, or 43%. Of 56 patients with cancer of the bladder, bone involvement was present in 6 cases, or 10%, but in only 1 instance were true metastases observed. In the other cases, the pelvis was invaded by direct extension from the primary tumor. In this series, 4 cases, or 7%, showed intrathoracic involvement.

Intensive radiotherapy relieves pain in a large number of patients with skeletal metastases from cancer of the kidney and prostate. Irradiation is often ineffectual in pulmonary metastases from cancer of the urinary tract. Occasionally, however, there may be relief of cough and dyspnea, and, when pleural effusions are present, there is slower refilling of the chest following such treatment.—E. H. Q.

Malignant Renal Neoplasms. Abeshouse, B. S., and Weinberg, T. [Sinai Hosp., Baltimore, Md.] *Arch. Surg.*, **50**: 46-55. 1945.

A series of 63 cases of renal cancer is analyzed for data on frequency, symptoms, and prognosis.—W. A. B.

An Application of Staging in the Removal of Difficult Wilms' Tumors. Sugarbaker, E. D. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] *Ann. Surg.*, **120**: 901-910. 1944.

The author accepts the evidence in the literature that primary nephrectomy for Wilms' tumor is more desirable than preliminary irradiation followed by surgery and reports a case in which the tumor was removed in two stages with an interval of 6 days between operations. Roentgen therapy was given after operation. The patient was alive 2½ years postoperatively. A review of the pertinent anatomy is included in the paper.—W. J. B.

Bilateral Renal Carcinoma. Hanley, H. G. [Hillingdon County Hosp., Middlesex and St. Paul's Hosp., London, England] *Brit. J. Surg.*, **32**:399-402. 1945.

This is the tenth case to be reported in the literature. There was nothing to show that the two tumors were not independent in origin.—E. L. K.

Carcinoma of the Bladder (Total Cystectomy). Ritter, J. S., and Kramer, S. E. [New York, N. Y.] *Urol. & Cutan. Rev.*, **49**:532-534. 1945.

The authors have discontinued radium implantation in their treatment of bladder carcinoma, and have become dissatisfied with the results of partial cystectomy because of the large recurrence rate. They advocate fulguration and total cystectomy. Two cases are presented.—V. F. M.

Leiomyosarcoma of the Urinary Bladder With Gynecological Pathology. Lash, A. R. [Univ. of Illinois, and Michael Reese Hosp., Chicago, Ill.] *Urol. & Cutan. Rev.*, **49**: 431-432. 1945.

The author finds in the literature only 15 cases of leiomyosarcoma of the bladder and adds 1 more.—V. F. M.

Sarcoma of the Bladder. Tahara, C., Burt, K. L., and Hess, E. [St. Vincent's Hosp., Erie, Pa.] *Urol. & Cutan. Rev.*, **49**:534-538. 1945.

An instance of fibromyxosarcoma of the bladder in a child of 6 years is described and illustrated. The lesion is rare. One of the authors has a child patient surviving 7 years after partial removal of a plexiform neurofibrosarcoma involving the prostate, bladder, and adjacent tissues.—V. F. M.

Primary Carcinoma of the Female Urethra. Hess, E. [Erie, Pa.] *Pennsylvania M. J.*, **48**:1150-1155. 1945.

Cancer of the female urethra is a preventable disease if the condition of granular urethritis is recognized and adequately treated. Unfortunately, according to the author, little attention is paid to the urethra by women or their physicians even after symptoms develop. The majority of hyperplastic conditions of the female urethral mucosa usually occur in women between 40 and 70 years old, with a history of genital infection of long standing, with or without injury to the birth canal.

The percentage of malignancies in mucosal hyperplasias is much larger than ordinarily considered, approximately 33%. When glandular metastases are present, which usually occur late, the prognosis is bad. With extensive infiltration along the anterior vaginal wall, the prognosis is better if transplantation of the ureters to skin or bowel is followed by total cystectomy and the removal of a large flap of the anterior vaginal wall. Most of the cases do not respond to radium or x-ray therapy alone. The author's procedure is to remove thoroughly the lesion plus a large area of normal tissue by means of the electric loop and then to refer the patient to the roentgenologist for treatment by radium needle therapy. This is indicated in the drawings representing the various operative procedures.—J. L. M.

Urinary Tract Changes with Benign Pelvic Tumors. Chamberlin, G. W., and Payne, F. L. [Reading, Pa., and Univ. of Pennsylvania, Philadelphia, Pa.] *Radiology*, **42**:117-121. 1944.

In a study of 96 patients with benign pelvic tumors, 66 showed roentgen evidence of ureteral obstruction, or ureteral displacement or both, the majority (47.9%) showing obstruction. The tumors were myomas, or ovarian cysts or both. The position of the tumor in relation to the pelvic ureter seemed more important than its size. Pathologic changes of the urinary tract ranged from slight dilatation of the upper ureter to a rare case of markedly widened, elongated ureter and hydronephrosis. In no case did a complete absence of function occur. Postoperatively the urinary tracts returned to normal. The changes were considered to be due to mechanical pressure. —R. E. S.

Oral Cavity and Upper Respiratory Tract

Rhinology in Children, Résumé of and Comments on the Literature for 1944. Wishart, D. E. S. [Toronto, Canada] *Laryngoscope*, **55**:381-426. 1945.

This is a general article with 2 pages devoted to the subject of tumors, and 4 papers on this subject are reviewed. The review includes case reports on an ossifying fibroma of the superior maxilla, ivory osteoma of the left ethmoid, small round-cell sarcoma of the ethmoid, and a congenital nasopharyngeal dermoid. There are no illustrations.—A. M.

Clinicopathologic Conference. Transition Cell Carcinoma of the Nasopharynx. Report of a Case with Involvement of All Twelve Cranial Nerves. Flynn, J. E. [Army and Navy Gen. Hosp., Hot Springs Nat. Park, Ark.] *J. Iowa M. Soc.*, **36**:9-11. 1946.

The case is reported because of metastastic involvement of nervous tissue. The tendency of this type of primary tumor is to metastasize early to the regional lymph nodes and then into the blood stream. The high degree of radiosensitivity of these tumors and their implants is not associated with a proportionate degree of radiocurability.—M. E. H.

Hamartoma of Tongue. STAMM, C., and TAUBER, R. [Philadelphia, Pa.] *Laryngoscope,* **55**:140-146. 1945.

This tumor, in a newborn girl, was discovered because it produced difficulty in swallowing. It was readily removed with a snare and on histological examination was found to be lined by well differentiated squamous epithelium and to contain various well differentiated tissues that showed no evidence of "proliferative activity." There is a brief discussion of hamartomas and of the embryological origin of these tumors of the tongue. The differential diagnosis is briefly considered. There is a bibliography of 18 papers and there are 3 drawings showing the gross and microscopic characteristics as well as the embryology of tongue development.—A. M.

Some Interesting Lesions of the Tongue. SAGE, R. A. [Indianapolis, Ind.] *Arch. Otolaryng.,* **43**:122-133. 1946.

Sketchy outline of some lesions of the tongue.—C. R. N.

A Review of the Available Literature on the Larynx and Laryngeal Surgery for 1944. ORTON, H. B. [Newark, N. J.] *Laryngoscope,* **55**:33-51. 1945.

In this general article 26 papers are briefly reviewed, of these 14 relate to neoplasms. The editor adds a few comments of his own. On the subject of papilloma of the larynx in children, he emphasizes the value of biopsy since he has seen papillary epidermoid carcinoma of the larynx in a child of 12. He states that in surgery for carcinoma of the larynx he removes the hyoid bone, larynx, and preepiglottic space in all laryngectomies, and that surgery still plays the most important part in the cure of carcinoma of the larynx. There are no illustrations.—A. M.

Cancer of the Larynx. Five Year Results of Concentration Radiotherapy. CUTLER, M. [Chicago Tumor Inst., Chicago, Ill.] *Arch. Otolaryng.,* **43**:315-330. 1946.

Modern radiation therapy is an increasingly effective method of treatment for laryngeal cancer. This statement is based on the analysis of 118 consecutive cases of cancer of the larynx treated by concentration radiotherapy (large doses over a comparatively short time—18 days to one field—total dose 6,000 to 6,500 r) over 3 years after treatment. Larynxes with intrinsic carcinoma too advanced for laryngofissure and requiring total laryngectomy have been cured by irradiation. There were 42% five year cures (18 of 47 cases) and 39% three year cures (43 of 118 cases).—C. R. N.

Cancer of the Larynx. TUCKER, G. [Philadelphia, Pa.] *Pennsylvania M. J.,* **48**:809-811. 1945.

A general discussion.—J. L. M.

SALIVARY GLANDS

Adenolymphoma of the Salivary Glands. MCNEELY, R. G. D. [Univ. of Toronto and Toronto Gen. Hosp., Toronto, Canada] *Canadian M. A. J.,* **54**:124-129. 1946.

Five new cases of the tumor are reported making the total number now on record 108. The most logical explanation as to the origin seems to be that the epithelial elements of the tumor are derived from the salivary duct epithelium, and the lymphoid tissue occasionally found about the interlobular ducts may give rise to the lymphoid tissue of the adenolymphoma and at times of salivary adenomas.—M. E. H.

Mikulicz's Syndrome: Report of a Case with Associated Pulmonary and Gastric Lesions. MILLER, J. R., and EUSTERMAN, G. B. *Proc. Staff Meet., Mayo Clin.,* **19**:425-431. 1944.

This case of Mikulicz's disease was a chronic lymphoblastomata with a low degree of clinical malignancy. The histologic pictures in an orbital nodule removed for biopsy were almost identical with the histopathologic findings originally described by Mikulicz. The authors question the assumption that Mikulicz's original case was a benign self-limited process.—J. L. M.

INTRATHORACIC TUMORS—LUNGS—PLEURA

Surgical Management of Large Tumors of the Neck. DIXON, C. F., and BENSON, R. E. [Mayo Clin., Rochester, Minn.] *Am. J. Surg.,* **69**:384-390. 1945.

Two cases of large neck tumors are presented, one a cervical and intrathoracic goiter, and the other a huge mixed tumor of the parotid. In the first case, the importance of an adequate incision, excision of redundant skin at the beginning of the procedure, with the construction of skin flaps, and drainage of the dead space left by removal of the gland, is stressed. In the second case, a parotid tumor, the preservation of the internal, external, and common carotid arteries, the vagus nerve and the ansa hypoglossi presents the primary difficulty, and early isolation of these structures is advised. This patient received postoperative irradiation because of the high incidence of malignancy in this type of tumor.—W. A. B.

Pulmonary Alveolar Adenomatosis in Man. Is This the Same Disease as Jaagsiekte in Sheep? WOOD, D. A., and PIERSON, P. H. [San Francisco, Calif.] *Am. Rev. Tuberc.,* **51**:205-224. 1945.

This unusual condition is described in a woman of 57 who was subjected to a lobectomy for symptoms of several years standing referable to the chest. A detailed description of the tumor is included with photomicrographs. When the patient died about 6 months later parts of the lung were preserved in glycerine and in the frozen state. Additional findings were a carcinoma of the cervix and bizarre metaplastic changes suggestive of neoplasia in the kidney, pancreas, and thyroid. Attempts to demonstrate the presence of a virus in the lung are now in progress, since the lesion closely resembles Jaagsiekte in sheep, which is thought to be an infectious process. Since the condition is rarely recognized even at autopsy, this is a rare opportunity for study of a possible virus that may produce definite hyperplasia in the lung.—E. E. S.

Report of an Unusual Case of Mediastinal Tumor. CLAYMAN, S. G. [San Haven, N. D.] *J. Lancet,* **66**:184-187. 1946.

Seven distinct severe diseases were found at the autopsy of this patient. The malignant tumor proved to be a prickle cell carcinoma of the lung with metastasis to the periaortic nodes.—M. E. H.

A Teratoid Tumor of the Chest: A Case Report. DANN, D. S., LOCKWOOD, H., NEIBLING, H. A., and WALKER, J. W. [Kansas City, Mo.] *Radiology,* **44**:585-587. 1945.

A case report of a benign tumor of the anterior mediastinum is presented.—R. E. S.

Multiple Pulmonary Hemangiomata. MAKLER, P. T., and ZION, D. [Graduate Hosp. Univ. of Pennsylvania, Philadelphia, Pa.] *Am. J. M. Sc.*, **211**:261-267. 1946.

A report is given of a patient with polycythemia, cyanosis, clubbing of fingers, and slight dyspnea on exertion in which the findings pointed to the presence of multiple pulmonary hemangiomas as the causative agent.—M. T.

Bronchogenic Carcinoma. Diagnosis by Cytologic Study of Bronchoscopically Removed Secretions HERBUT, P. A., and CLERF, L. H. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *J. A. M. A.*, **130**: 1006-1012. 1946.

Significant signs and symptoms are often not present until late in carcinoma of the lung. The excellent results obtained by examining vaginal smears for cancer cells in carcinoma of the cervix and uterus led to the examination of bronchial secretions for tumor cells in suspected cases of carcinoma of the lung. Cancer cells were demonstrated in 22 of 30 cases or 73%. A positive morphological diagnosis from a study of tissue removed endoscopically was obtained in 11 cases or 36% in the same series. The examination of bronchial secretions for tumor cells is superior to examination of the sputum. The method described may be an adjunct to the means already employed in the diagnosis of pulmonary carcinoma. It is particularly useful in early cases, in those in which the tumor is located at the periphery of the lung or in the upper lobe.—M. E. H.

College of Medicine Clinicopathologic Conference. *J. Iowa M. Soc.*, **36**:241-245. 1946.

The case presented was one of bronchogenic carcinoma of the apical portion of the right lung, with widespread metastases. In the discussion, a review of 200 clinical cases seen at the State University of Iowa Hospitals and 50 autopsies from the same source is presented.—M. E. H.

Primary Lung Tumors. ADAMS, R. [Lahey Clin., Boston, Mass.] *J. A. M. A.*, **130**:547-553. 1946.

One hundred and sixty-four cases of microscopically proved primary lung tumors are reported from the Lahey Clinic as occurring during the 15 year period from 1930 to 1944. Seven cases were nonmalignant: 1 myxochondroma and 6 bronchial adenomas. Primary carcinoma of the lung was proved in 157 cases. The frequency of cancer of the lung, compared with all other cancer, is between 6 and 8%. The age incidence follows that of malignant disease in general, being greatest between 40 and 50 years. There was a male preponderance of 128 to 29. The 5 most common symptoms of cancer of the lung encountered in this group in order of occurrence were found to be cough, chest pain, sputum, hemoptysis, and wheezing respiration. The symptomatology and diagnostic features of the group of 7 nonmalignant tumor cases were similar to those of the cancer series. Forty-nine (31.4%) of the 157 cases of pulmonary carcinoma were submitted to surgical resection. Of the resected group, there were 8 hospital deaths (16.3%). Twenty-three deaths subsequently occurred from recurrence of disease (46.9%). Four patients are living with evidence of recurrence (8.1%). Fourteen patients (28.5% of the 49 with resections, and 8.9% of the 157) are alive and well at the time of writing with a chance for 5 year survival. The longest survival is 9 years. Technical procedures have reduced the hospital mortality in the last 4 years in 30 consecutive resections to 3.3%.—M. E. H.

Surgical Aspects of Bronchiogenic Carcinoma. BRINDLEY, G. V., JR. *Proc. Staff Meet., Mayo Clin.*, **19**:361-369. 1944.

This report of the surgical aspects of bronchiogenic carcinoma consists of a study of 90 cases of bronchiogenic carcinoma in which exploratory thoracotomy was performed. The period covered by this series is from 1925 through 1943. In 45 cases the lesions were considered suitable for surgical extirpation at the time of exploration, a resectability rate of 50% of the cases selected for surgical intervention. The author briefly discusses the following clinical aspects of bronchiogenic carcinoma: sex and age, symptoms and diagnosis, pathology, preoperative measures, anesthesia, operative technic, and results of treatment.—J. L. M.

Lateral Cervical (Bronchial) Cysts and Fistulas. NEEL, H. B., and PEMBERTON, J. DE J. [Mayo Clin., Rochester, Minn.] *Surgery*, **18**:267-286. 1945.

A study was made of 319 cases in which operation was performed for lateral cervical cysts or fistulas. These lesions have a distinct pathologic appearance. The authors believe that the diagnosis of "bronchial carcinoma" is probably erroneous, and that this lesion is probably secondary to an undiscovered primary lesion in the nasopharynx.—W. A. B.

Clinical Features of Bronchiogenic Carcinoma. TINNEY, W. S., *Proc. Staff Meet., Mayo Clin.*, **19**:354-356. 1944.

From 1925 to 1942 inclusive, the clinical diagnosis of bronchiogenic carcinoma was made in 948 cases at the Clinic. This report is limited to the 448 cases in which the diagnosis was confirmed by microscopic examination of tissue obtained from the primary lesion through the bronchoscope, at surgical exploration or at necropsy.

The sex incidence showed that bronchiogenic carcinoma occurred in males about 5 times as often as in females. The disease was found to be much more frequent among patients who were past the age of 40 than in the earlier age groups. In this series 88% of the patients were more than 40 years old. The highest incidence was found in the sixth decade. The symptoms of the disease and the factors responsible for the usual delay in diagnosis are discussed.

Postmortem examination was performed in 70 cases, and locations of the metastatic lesions are tabulated. In 315 cases (70%) in which an adequate follow-up study was available, the average duration of life after the diagnosis was established was 6 months. The average duration of life from the onset of symptoms was 14½ months. The delay in diagnosis is the most significant factor in the poor prognosis of bronchiogenic carcinoma. In this series the prognosis was not influenced by the type or grade of tumor.—J. L. M.

Primary Mesothelioma (Endothelioma) of the Pleura. PIATT, A. D. [Newark Hosp., Newark, Ohio] *Am. J. Roentgenol.*, **55**:173-180. 1946.

A complete case report is presented of this rare condi-

tion, with radiographs and photographs of gross and microscopic lesions.—E. H. Q.

Pulmonary Tuberculosis and Pulmonary Carcinoma Each Masquerading as the Other. Fair, E. C., and Clagett, O. T. *Proc. Staff Meet., Mayo Clin.*, **20**:342-345. 1945.

Two case reports are presented.—J. L. M.

Expectoration of Carcinomatous Tissue without Localizing Signs in Four Subsequent Years; Pneumonectomy: Report of Case. Sampher, R., and Clagett, O. T. *Proc. Staff Meet., Mayo Clin.*, **20**:341-342. 1945.

A case report in which pneumonectomy was performed. —J. L. M.

Miliary Carcinosis of the Lungs Secondary to Primary Cancer of the Gastrointestinal Tract. Culver, G. J. [Buffalo Gen. Hosp., Buffalo, N. Y.] *Am. J. Roentgenol.*, **54**:474-482. 1945.

Miliary carcinosis of the lungs is not common, but when it occurs it is important that it be recognized. Four cases are reported with roentgenograms showing the typical appearance. Miliary tuberculosis and silicosis must be considered in the differential diagnosis.—E. H. Q.

Aplastic Anemia Terminated by Removal of a Mediastinal Tumor. Humphreys, G. H., and Southworth, H. [Presbyterian Hosp., and Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Am. J. M. Sc.*, **210**:501-510. 1945.

Case report. The nature of the mediastinal tumor is in doubt.—J. G. K.

Gastrointestinal Tract

Carcinoma of the Oesophagus: Resection and Oesophago-Gastrostomy. Thompson, V. C. [London, England] *Brit. J. Surg.*, **32**:377-380. 1945.

Description of a case.—E. L. K.

An Operation for Removal of Carcinoma of the Oesophagus with Presternal Oesophago-Gastrostomy. Taylor, H. [The London Hosp. and King George Hosp., London, England] *Brit. J. Surg.*, **32**:394-399. 1945.

Descriptions of cases.—E. L. K.

Oesophagectomy for Carcinoma. Restoration of Function by an Ante-Thoracic Jejunal Graft. Thompson, V. C. *Proc. Roy. Soc. Med.*, **39**:310-311. 1946.

Description of a case.—E. L. K.

College of Medicine Clinicopathologic Conference January 14, 1946. Clinical Discussion. Peterson, F. R. [General Surgery] and Plass, E. D. [Obstetrics and Gynecology] *J. Iowa M. Soc.*, **36**:157-161, 171. 1946.

Case was one of adenocarcinoma of the stomach with pyloric obstruction with direct extension to the pancreas and metastases to regional lymph nodes. Pregnancy and mild tubular nephritis complicated the clinical picture.—M. E. H.

Is the Incidence of Gastric Cancer Overrated? Mulsow, F. W. [Cedar Rapids, Ia.] *J. Iowa M. Soc.*, **36**:194-195. 1946.

The death certificates reported to the City Hall in Cedar Rapids revealed 125 cases of cancer of the stomach among 1,091 deaths from cancer and other malignant conditions during the past 11 years. An incidence of 5.8% (358 cases) of diagnosed cancer of the stomach among 6,136 cases of cancer were found at the Tumor Clinic of the State University of Iowa during the period July 1, 1939, to June 30, 1945. The 5 year period from January 1, 1940, to December 31, 1944, revealed 129 cases of stomach cancer (6.7%) among 1,916 cases of all cancers studied at the Presbyterian Hospital of Chicago. Among 1,462 primary admissions of cancer patients to the Hines Hospital during 1943, cancer of the stomach was found in 81 or 5.5%.

It would seem from the present study that cancer of the stomach is obviously not the most common form of cancer and lacks proof of being the cause of 20 to 30% of all cancer deaths, as is found in most text books concerning the incidence of cancer of the stomach.—M. E. H.

Perforated Gastric Carcinoma Treated by Resection: Report of Case. Jarboe, J. P., and Pratt, J. H. *Proc. Staff Meet., Mayo Clin.*, **20**:446-448. 1945.

The authors report a case of perforated carcinoma of the stomach complicated by subdiaphragmatic abscess. Operation was not performed until 22 days after the perforation occurred and the patient's condition was extremely critical during this period. Although gastric resection was performed under most unfavorable circumstances, the response of the patient was satisfactory.—J. L. M.

Gastric Leiomyoma. Vier, H. J. [White Plains, N. Y.] *N. Y. State J. M.*, **45**:2084. 1945.

Gastric leiomyoma is a rare lesion and in this respect may not cause symptoms until markedly advanced, as evidenced by the patient presented. The significant feature of this case is that the patient remained entirely comfortable and symptom-free prior to the onset of his hemorrhage, which was due to ulceration of the neoplasm.—J. L. M.

Determination of Operability of Cancer of the Stomach with the Abdomen Opened. Lahey, F. H. [Lahey Clin., Boston, Mass.] *Lahey Clin. Bull.*, **4**:194-196. 1946.

Inspection and palpation of the lesion itself as well as of the adjacent structures plus the examination of the jejunal and pelvic fossae will enable one to tell before surgery the type and extent of the operation necessary. It is also well to have in mind these examination procedures, which one must follow when the abdomen is open and the lesion exposed, in order to determine the operability of the tumor.—M. E. H.

Case Reports of Barnes Hospital. Clinical and Postmortem Records Used in Weekly Clinicopathologic Conferences at Barnes Hospital, St. Louis. Wood, W. B., Jr., and Moore, R. A. [St. Louis, Mo.] *J. Missouri M. A.*, **43**:252-258. 1946.

Case 87 presented the problems associated with metastatic leiomyosarcoma in the liver, pancreas, lower lobe of the right lung, the upper lobe of the left lung, the right kidney, gall bladder, urinary bladder and mesentery. The history revealed that a pedunculated tumor of smooth muscle of the stomach had been removed at the time of an exploratory laparotomy, 10 months prior to death.—M. E. H.

The Surgical Care of the Operable Malignant Lesions of the Stomach. Hunt, C. J., and Hunt, P. F. [Research Hosp., and Kansas Univ. Med. Sch. Kansas City, Mo.] *J. Missouri M. A.*, **43**:21-23. 1946.

Preoperative evaluation of the patient's physiologic resources, and local anatomic preparation of the stomach for surgical approach are essential to the success of the operation. The anesthetic and surgical procedures are discussed as well as certain preferences on points of technical importance. Essential measures of postoperative management are outlined.—M. E. H.

Gastrostomy: The History of its Development, Indications and Contraindications. Thorek, M. [Chicago, Ill.] *Rev. Gastroenterol.*, **12**:347-352. 1945.

The history of the development of gastrostomy is reviewed. Indications for operation include certain diseases of the pharynx and larynx (and those of cervical origin involving these passages), obstructive lesions of the esophagus and mediastinum, and of the gastric cardia when extirpation of a tumor is not contemplated. Absolute and relative contraindications are discussed. The operation should be performed before the patient's physical condition has become poor, and attention should be given to maintaining a well-balanced and enriched diet.—L. J. D.

Intussusception in Adults. Iason, A. H. [Brooklyn, N. Y.] *Surgery*, **18**:457-466. 1945.

The most common cause for intussusception in adults is a benign or malignant tumor. Enteric intussusception is the only form that occurs in adults with greater frequency than in infants, but the majority of instances in adults are of the enterocolic variety. Six cases are presented, in 4 of which the apex of the intussusception was formed by the tumor; in the fifth case there was a carcinoma in the intussuscipiens and not in the intussusceptum, and in the sixth case no tumor was found.—W. A. B.

Small Bowel Stricture Following Irradiation Therapy for Carcinoma of the Fundus Uteri. Saltzstein, H. C. [Detroit, Mich.] *Surgery*, **18**:556-560. 1945.

The patient was a 49 year old woman who had received prolonged x-ray therapy for carcinoma of the fundus of the uterus. Abdominal symptoms started during treatment, and progressed after several months to episodes of severe abdominal cramps with vomiting. One attack of incomplete small bowel obstruction was relieved after the use of a Miller-Abbott tube; 2½ months later, an exploratory laparotomy was done for another attack of intestinal obstruction. The terminal ileum for 12 inches was thickened and there was a dense adhesion causing kinking, which was relieved, with satisfactory recovery of the patient.—W. A. B.

Melanoma of Small Intestine. Wade, B. N. [Portland, Oregon] *Northwest Med.*, **44**:388. 1945.

It is deemed unlikely that in the few reported cases of melanoma involving the small intestine the tumors were primary in this site since melanoblasts have not been identified in the intestinal wall. However, in the case described here, physical examination revealed no other growth. The patient died following symptoms of cerebral metastases, but autopsy was not performed and confirmation was lacking. The tumor was identified by histologic study following resection.—E. E. S.

Lymphosarcoma of the Small Intestine. Berman, H., and Mainella, F. [Unity Hosp., Brooklyn, N. Y.] *Am. J. Surg.*, **70**:121-125. 1945.

The incidence of lymphosarcoma of the intestine, as determined from a review of the literature, is extremely rare. The case reported is one occurring in a girl of 18 years, who showed metastases to the glands. She was alive and well 5 years after operative removal of the involved portion of the jejunum and the glands. X-ray therapy had been avoided because of the danger of sterilization. This case is one of the few in which a 5 year survival has been reported.—W. A. B.

Benign Tumors of the Duodenum. Hoffman, B. P., and Grayzel, D. M. [Jewish Hosp. of Brooklyn, Brooklyn, N. Y.] *Am. J. Surg.*, **70**:394-400. 1945.

In a group of 64,300 surgical specimens and 4,480 autopsies, the authors found 58 tumors of the small intestine; 33 were benign. Of the latter, 14 occurred in the duodenum, an incidence of 42.4%; of the malignant tumors, 4, or 16%, were in the duodenum. Thirteen cases in which the benign duodenal tumor was an incidental finding at autopsy, and a 14th case in which it was removed at the time of operation for a common duct stricture, are discussed. These are tabulated, showing a total of 6 pancreatic rests, 4 adenomas, 1 lipoma, 1 fibroma, 1 lymphangioma and 1 gastric rest.—W. A. B.

Primary Malignant Disease of the Small Intestine. Shallow, T. A., Eger, S. A., and Carty, J. B. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *Am. J. Surg.*, **69**:372-383. 1945.

A case of primary jejunal carcinoma producing a contact fistula with the terminal ileum is presented and 38 previous cases of primary malignancy in the small intestine are reviewed. From this and other series, the incidence of both carcinoma and sarcoma in the small bowel is found to be 0.1% of all general autopsies, as compared with 3.6% for the large bowel. In order of frequency of appearance, the types of carcinoma are stenosing, infiltrating or ulcerative, and polypoid. Diagnosis in 17 out of 29 of these cases was aided by the x-ray examination; in 75% of this series, there was a palpable tumor. In this group, a variety of surgical procedures was used, depending on the condition of the patient and the location of the lesion. All of the jejunal lesions, 5 in number, were resected, followed by primary anastomosis, either side-to-side or end-to-end. The operative mortality was 44% of the 27 cases, and was highest in the duodenal group.—W. A. B.

Roentgenologic Examination of the Small Intestine. Good, C. A. [Chicago Roentgen Society, Regular Meeting March 14, 1946] *Proc. Inst. Med. Chicago*, **16**:202-203. 1946.

Methods used for the roentgenologic examination of the small intestine are of two types: roentgenography and roentgenoscopy. Benign and malignant tumors can be more easily identified if frequent roentgenoscopic observations are made at frequent intervals after the administration by mouth or by tube of a barium mixture in water. Roentgenograms can be made to confirm any lesion located in this manner. Meckel's diverticulum and inflammatory

lesions can also be identified by the use of this method.—M. E. H.

Operative Results of Surgery of the Colon for Neoplastic Disease. Pierpont, R. Z., Peterson, F. R., and Dulin, J. W. [Univ. Hosps., Iowa City, Ia.] *Surgery*, **18**:541-546. 1945.

In a series of 224 patients, observed between 1937 and 1944, on whom exploratory laparotomy was carried out for neoplasms of the colon, 117 had resection of the colon, and 81 patients had only palliative operations. Of the total, 142 patients had lesions of the left side and 82 had right sided lesions. For the right sided lesions, the procedure of choice was a one stage resection with immediate ileocolostomy. The mortality in this group was 29.2%. Three types of procedure were used for lesions of the left side: 33 having modified Mikulicz procedures, operative mortality of 15.1%, 21 with resection and immediate end-to-end anastomosis, mortality of 19%, and 14 having the Hartman procedures, with no deaths. Sepsis was the major cause of death in all groups. —W. A. B.

Carcinoma of Colon and Rectum. Miller, G. I. [Brooklyn, N. Y.] *M. Rec.*, **158**:601-604. 1945.

The article is a very general discussion of the nature and behavior of tumors of these regions. The frequent occurrence of multiple neoplasms is noted, and mention is made of the influence of heredity on metastasis and localization. There is detailed discussion of the surgical procedures advised for removal of the growth, and supportive measures are urged to reduce the operative mortality.—E. E. S.

Acute Colonic Obstruction Secondary to Carcinoma of the Sigmoid with Gangrene of an Extensive Segment of the Large Bowel. Kremen, A. J. [(M.C.) U.S.A.] *Surgery*, **18**:335-338. 1945.

The case reported is that of a 43 year old white soldier who suddenly developed symptoms of acute intestinal obstruction. He had had one previous episode of abdominal cramps 5 weeks earlier. There was an annular, constricting, ulcerated adenocarcinoma of the sigmoid and the entire colon from cecum to sigmoid was gangrenous. Resection of the colon with ileosigmoidostomy was done with a favorable result.—W. A. B.

Sigmoidoscopy in Diagnosis and Treatment. Yeomans, F. C. [New York, N. Y.] *Rev. Gastroenterol.*, **12**: 38-42. 1945.

The indications for and technic of sigmoidoscopy are described. Data obtained include the presence or absence of abnormal conditions of the mucosa, of ulcerative lesions, and of benign or malignant tumors, single or multiple. Studies by swab or biopsy may be made on areas exposed by the sigmoidoscope; treatment may be applied, and follow-up studies carried out. Seven illustrative cases are cited.—L. J. D.

Evolution of Sphincter Muscle Preservation and Re-Establishment of Continuity in the Operative Treatment of Rectal and Sigmoidal Cancer. Bacon, H. E. [Temple Univ. Sch. of Med., Philadelphia, Pa.] *Surg., Gynec. & Obst.*, **81**:113-127. 1945.

Clinical discussion with illustrations of operative procedure.—J. G. K.

Carcinoma of Sigmoid and Rectosigmoid Involving Urinary Bladder. Dixon, C. F., and Benson, R. E. [Mayo Clin., Rochester, Minn.] *Surgery*, **18**:191-199. 1945.

Sixty-four cases in which early invasion of the urinary bladder by carcinoma of the sigmoid or rectosigmoid occurred are reviewed. Diagnosis of urinary bladder involvement may be aided by symptoms of urinary discomfort, hematuria, pyuria, or even passage of feces by the urethra after formation of a rectovesical fistula. Cystoscopy reveals the extent of the lesion. Forty patients were treated by extensive resection, with removal of a portion of the bladder wall; in this group there were 7 operative deaths. Palliative operations were done in 22, and in 8 of these there were metastases to the liver, lung or other distant organs. In 7 cases the debility of the patient was such that resection was considered inadvisable, and in the remaining 7, the lesion was nonresectable because of extensive spread to other pelvic organs.—W. A. B.

Cancer of the Rectum and Rectosigmoid. Murdoch, R. L. [Univ. of Oklahoma Med. Sch., Oklahoma City, Okla.] *J. Oklahoma M. A.*, **38**:239-242. 1945.

A summary of 50 consecutive radical resections of the rectum and rectosigmoid colon is presented. Six types of operation are included, with a discussion of preoperative preparation, supportive measures, and anesthesia.—E. E. S.

The Diagnostic and Prognostic Signs in Cancer of the Colon and Rectum. Lahey, F. H. [Lahey Clinic, Boston, Mass.] *Lahey Clin. Bull.*, **4**:162-166. 1945.

Carcinoma of the colon and rectum produces early symptoms of blood in the stool, altered bowel function, and abdominal cramps or pain in 97.7% of cases. Every patient having one or more of these three symptoms should have a complete and thorough examination of the colon by barium enema, contrast enema, and sigmoidoscope examination. Prompt examination after the onset of these complaints would result in the increased operability of cancer of the colon and rectum as well as their nonrecurrent rate. The mortality due to these lesions would be lowered. Polyps of the rectum and colon have been found in at least 3% of all people, and the tendency for these lesions to give malignant degeneration is well known. The question of the advisability of removal of the growths is discussed.—M. E. H.

Radical Resection of Carcinoma of Rectum and/or Rectosigmoid in Continuity with Retention of the Anal Sphincter. Wilensky, A. O. [New York, N. Y.] *J. M. Soc. New Jersey*, **42**:328-331. 1945.

The author, presenting 2 cases, emphasizes the advantage of retaining the anal sphincter in the radical operation for removal of a carcinomatous lesion of the rectum or rectosigmoid. This is possible in all cases in which there is present at least 3 inches of normal terminal bowel distal to the lower margin of the tumor.—M. E. H.

Colloid Carcinoma in Anal Fistula: Report of Two Cases. Ducassi, E. R., and Smith, N. D. *Proc. Staff Meet., Mayo Clin.*, **20**:57-60. 1945.

Two cases have been observed in which colloid carcinomas of long standing have developed in an anal fistula. The unusual location of the sac or tumor and the rarity

of such cases have led the authors to record these findings. —J. L. M.

Polyps of the Appendix. White, J. W., and Ellis, R. C. *Am. J. Surg.*, **70**:389-393. 1945.

The literature is reviewed and a new case added to the 21 previously reported. The patient was a man 33 years old, who had had symptoms and findings of ulcerative colitis for 11 years prior to the present illness. At this time he developed acute appendicitis, and the resected appendix showed a polyp obstructing its lumen, with secondary acute purulent appendicitis. The authors feel that the incidence of polyps in the appendix is higher than indicated by the cases reported, and that more would be revealed by careful examination of all appendices removed.—W. A. B.

Submucous Lipomas of Colon with Special Reference to Acute and Chronic Intussusception. Lazarus, J. A., and Marks, M. S. [New York, N. Y.] *Am. J. Surg.*, **70**:114-117. 1945.

Large submucous lipomas of the colon are associated with chronic or acute symptoms of abdominal discomfort. A case illustrating the acute symptoms is presented; in this 65 year old patient the tumor formed the apex of an intussuscepted portion of bowel. At operation, the intussusception was reduced and the tumor containing portion of the colon exteriorized. Resection of the tumor was performed after 5 days, and a Mikulicz closure of the colostomy several months later. Colostomy and extirpation of the lipoma is considered the best procedure when the diagnosis of the tumor is established.—W. A. B.

Technique and Results of Primary and Secondary Pull-Through Operation After Removal of Tumors of the Rectum and Rectosigmoid. Mandl, F. [Hadassah Univ. Hosp., Jerusalem, Palestine] *Surgery*, **18**:318-335. 1945.

The operative technic, which is described in detail, consists briefly in pulling the tumor through a sacral incision, with preservation of the anal sphincter. The secondary pull-through operation includes the establishment of an artificial prolapse from the sacral anus, by pressing exercises. This is resorted to in cases where the length of the bowel proves too short for the primary pull-through technic at the time of the radical operation. Six cases of the primary pull-through type are presented, and 3 cases in which the secondary type was utilized. In 75% of 16 cases continence was restored by either of these technics.—W. A. B.

Successful Resection for Stricture of the Rectosigmoid and Repair of Rectovaginal Fistula Following Radium Therapy for Carcinoma of Cervix: Report of Case. Black, W. A., and Waugh, J. M. *Proc. Staff Meet., Mayo Clin.*, **20**:87-89. 1945.

The authors give a general review and report one case in detail.—J. L. M.

Use of Omentum as a Pelvic Diaphragm Following the Resection of Perforating Carcinoma of the Rectosigmoid. Derbyshire, R. C. [Lovelace Clin., Albuquerque, N. M.] *Rocky Mt. M. J.*, **43**:388-389. 1946.

Case report. The use of the omentum to form a pelvic diaphragm was considered life-saving in the case presented. —M. E. H.

Peritoneum and Retroperitoneum

Primary Fibrosarcoma of the Great Omentum. Lawler, R. H., Fox, P. F., and Cohen, H. [Cook County Hosp., Chicago, Ill.] *Am. J. Surg.*, **69**:135-136. 1945.

A case report.—W. A. B.

Bone and Bone Marrow

Solitary Myeloma: Review of Sixty-one Cases. Gootnick, L. T. [Univ. of Chicago, Chicago, Ill.] *Radiology*, **45**:385-391. 1945.

Gootnick has collected 59 cases of "solitary" myeloma from the literature and reports 2 cases of his own. Of the total number of patients 44% were in the age group from 50 to 60. The x-ray appearance of the growth is either that of a cystic, trabeculated lesion resembling a giant cell tumor or an osteolytic lesion suggesting metastatic carcinoma. Roentgen therapy appears to be the treatment of choice; when it is used the duration of life extends several years beyond the average for multiple myeloma, and pain is relieved. The average duration of life in treated cases was 7 years from onset of symptoms and $3\frac{1}{2}$ years from the first admission. Some tumors remain solitary for many years and run a relatively "benign" course. Others spread rather rapidly and run the course of multiple myeloma.—R. E. S.

Relationship of the So-Called "Giant-Cell Tumor" to the Frontal Bone. Ferrington, E., and Mufson, J. A. [Mount Sinai Hosp., Milwaukee, Wis.] *Am. J. Clin. Path.*, **15**:529-533. 1945.

Case report with review of the literature.—J. G. K.

Xanthoma or Lipoid Granuloma of the Temporal Bone (Hand-Christian-Shuller Syndrome). Glatt, M. A. [Chicago, Ill.] *Arch. Otolaryng.*, **43**:110-121. 1946.

A case report.—C. R. N.

Embedment of a Vitallium Mandibular Prosthesis as an Integral Part of the Operation for Removal of an Adamantinoma. Winter, L., Lifton, J. C., and McQuillan, A. S. [Bellevue Hosp., New York, N. Y.] *Am. J. Surg.*, **69**:318-324. 1945.

The procedure described was used in one case, that of a 55 year old woman with a recurrent adamantinoma of the mandible.—W. A. B.

Capsular Osteoma of the Knee Joint. Report of Four Cases. Kautz, F. G. [New York Hosp., New York, N. Y.] *Radiology*, **45**:162-167. 1945.

Four cases of osteoma of the knee joint located beneath the patella are presented, 3 being proven at the time of surgery. Repeated trauma or an active athletic life may be mechanical factors in their formation.—R. E. S.

Progress in Orthopedic Surgery: Tumors of Bone and of Synovial Membrane. Meyerding, H. W. [Rochester, Minn.] *Arch. Surg.*, **51**:181-195. 1945.

A review of the recent literature on bone tumors and their classification is presented.—W. A. B.

So-Called "Subperiosteal Giant Cell Tumor." Present, A. J. [A. U. S.] *Radiology*, **44**:77-79. 1945.

A case report of a giant cell tumor of the shaft of the radius is presented.—R. E. S.

Book Reviews

HUMAN TORULOSIS. A Clinical, Pathological and Microbiological Study, With a Report of Thirteen Cases. Leonard B. Cox and Jean C. Tolhurst. Victoria, Australia: Melbourne University Press. 1946, xi + 149 pages. 67 illustrations. Price 25 shillings.

Human infections with the yeast-like organism, *Torula histolytica,* have presented difficult problems of diagnosis not only as to the nature of lesions of the skin, lungs, and lymph nodes, but also as to the differentiation between types of chronic and subacute meningitis, and between possible types of brain tumors. As the authors state: "The slow course, the occurrence of headache, the signs of intracranial pressure in many cases of torulosis have suggested the diagnosis of intracranial tumour." This relation of the organism to the formation of tumors is reflected in one of the specific names (*neoformans*) which have been given to it. It is now well known, however, that while the organism produces inflammatory and necrotic granulomas, it is not carcinogenic.

As the authors saw 13 cases of torulosis in Australia during the 8 year period of 1936 to 1944, and as cases have been reported from many parts of the world, they believe that the disease should no longer be considered rare. On the other hand, as fewer than 150 cases have been reported since 1895, when Busse and Buske reported a case which became known as the type case of European blastomycosis and American "torula meningitis," examples of the disease still excite interest as rarities. Beyond interest in the unusual, however, "the subject of torulosis, human and experimental, should be of interest," as the authors point out, "not only to the clinician, but to the pathologist and bacteriologist." Human infections present difficult problems of diagnosis in the differentiation between types of chronic or subacute meningitis, particularly in adults, and between possible types of brain tumor. The characteristics of the pathogenic yeast-like organism which causes the disease are still incompletely known. Relatively little is known about the mode of infection and the epidemiology of the disease.

The authors describe in considerable detail the 13 cases which they observed. For the convenience of the reader, who may wish to select features which are of particular interest to him, the subject is divided into the following main sections: historical introduction, case reports, torulosis of various systems, microbiology of torula histolytica, experimental torulosis, pathology of torulosis, laboratory diagnosis of torulosis, sources and routes of infection in torulosis, treatment of torulosis, summary, conclusions, and a bibliography of 134 references. There is a good index. The monograph is a valuable contribution to knowledge of torulosis.

S. Bayne-Jones

A.A.A.S. RESEARCH CONFERENCE ON CANCER. A Conference of Papers and Discussions Presented at the Summer Meeting of the Section on Chemistry of the American Association for the Advancement of Science at Gibson Island, Maryland, July 31-August 4, 1944. Edited by Forest Ray Moulton. Washington, D. C.: American Association for the Advancement of Science. 1945, x + 333 pages, with illustrations and tables. Price $4.00 to members of A.A.A.S., $4.50 to others.

As indicated in the title this volume is a record of the papers presented and edited reports of the oral discussions they stimulated at the 1944 A.A.A.S.-Gibson Island Research Conference on Cancer. There were 5 general chemical or biochemical aspects of cancer research represented on the program and incorporated in this volume: I. The Virus Approach, 6 principal papers, 4 supplementary notes, and the discussion (107 pages). II. Carcinogenesis, 6 principal papers and the discussion (62 pages). III. Enzymes, 5 principal papers and the discussion (83 pages). IV. Diet, 2 principal papers, 1 note, and the discussion (38 pages). V. Chemotherapy, 4 principal papers, 3 notes, and the discussion (43 pages). There were 42 contributors to the principal papers, 8 to the notes, and about 20 to edited oral discussions. The volume is generously illustrated and contains comprehensive references to the literature.

S. Bayne-Jones

SELECTED PAPERS FROM THE ROYAL CANCER HOSPITAL (FREE) AND THE CHESTER BEATTY RESEARCH INSTITUTE. Volume III. London: Royal Cancer Hospital (Free). 1941-42, viii + 345 pp., with illustrations.

In this volume there have been reproduced by the Replika Process 39 reports of clinical and experimental investigations of cancer as they were printed in a number of medical and scientific journals in England and in the United States during the years 1941 and 1942. The papers may be divided into three groups: I. Cancer surgery—1 paper on gastrectomy for gastric cancer. II. Cancer and radiation—18 papers on clinical, physical, and physiological studies of radiation. III. Cancer research—20 papers by the workers in the Chester Beatty Research Institute, with particular emphasis on chemical aspects of carcinogenesis.

The volume is notable for its content of important papers reporting further developments along the lines of fundamental research which have for many years been characteristic of the Chester Beatty Research Institute under the direction of Professor E. L. Kennaway. It is also a fine example of the continuation of British scientific productivity during the war.

Most of these papers have been abstracted individually in earlier issues of *Cancer Research.*

Ralph G. Meader

BIOLOGICAL ACTIONS OF SEX HORMONES. Harold Burrows. Cambridge: Cambridge University Press. 1945, 514 pages. Price 42s.

This book of reference is a concise summary of a very large literature and as such is not suitable for a detailed review. The author says in his preface that he felt that "a co-ordinated summary of experimental inquiries in this field might be useful. In pursuing the idea attention has been confined almost entirely to biological work performed in the laboratory; the ultimate possibility of applying the experience so gained for the benefit of man has been the leading motive. . . . . The author would like to regard his book as a tribute to the pioneers of sex-hormone physiology, with special regard to John Hunter (1728-93), the first and greatest of them." There is a chapter on "Factors in the Causation of Mammary Cancer" and the excellent index contains a large number of entries under the headings "Cancer" and "Tumours." There is a bibliography of over 2,000 references.

E. L. KENNAWAY

THE X-RAY TREATMENT OF ACCESSIBLE CANCER. By Waldron D. Smithers. London: Edward Arnold and Company. 1946, 147 pages, 102 illustrations. Price 40 s.

The author deals with apparatus employed and the technic of x-ray treatment of cancer of the skin, buccal cavity and cervix uteri. There is also a special chapter on the contact x-ray treatment of cancer of the rectum and bladder and other sites made accessible by surgical exposure. Considerable emphasis is rightly laid upon the biological aspects of the treatment of cancer by radiation and the importance of proper records and statistics. The book is well and copiously illustrated and contains an excellent bibliography.

The book is a valuable and much needed contribution to radiotherapeutic literature.

M. LEDERMAN

ROENTGEN DIAGNOSIS OF DISEASES OF THE GASTRO-INTESTINAL TRACT. John T. Farrell, Jr. Springfield, Ill.: Charles C. Thomas. 271 + x pages. Illustrated. 1946. Price $5.50.

This book, described by the author as a manual, is the result of the development of mimeographed outlines used in teaching in the Graduate School of Medicine of the University of Pennsylvania. The presentation of the material is unusual. It begins with a careful definition of terms. The topics are arranged in the form in which they appear in the Standard Nomenclature of Disease with the appropriate numerals. Some are discussed briefly, others are listed in outline form with the effect on the contour, motility, and position given in as few words as possible, and still others mentioned as in the Standard Nomenclature without additional comment. Some excellent suggestions as to procedure are given. The section on the esophagus is particularly good in this respect. The illustrations are well chosen and excellently reproduced, as is to be expected in books published by Thomas.

Apparently on account of the purely didactic nature of the book, very few references are made and a bibliography is not given.

This book, which covers the entire gastrointestinal tract in only 257 pages, is obviously intended as an introduction to the roentgen examination of the gastrointestinal tract, and can be used as a basis for demonstrations of films to students. The more advanced student will require more elaborate discussions, but will find many helpful suggestions in this manual. The outline could be used for a cross index of diseases of the gastrointestinal tract.

ROSS GOLDEN